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Note on the Occurrence and Inheritance of Pigmented Wool.

By R. B. Kelley, D.V.Sc., and H. E. B. Shaw, B.V.Sc.**

Wool has many uses as a textile fibre. For some of these the material is dyed with dark colours, for others pastel shades are used, and for still others the fibre is made up without dyeing. For all but the first of these, the wool must be homogeneous with regard to colour; dark fibres in otherwise white fleeces are a disadvantage.

Perusal of the literature and descriptions of native types suggest that originally all sheep fleeces were pigmented and that sheep now carrying white wool result from domestication and controlled breeding. This conception would explain the observed occurrence of pigmented and parti-pigmented sheep among selected wool-bearing flocks. The absence of pigment could be the effect of what are known genetically as inhibiting factors, while the pattern caused by discrete areas of pigmented and white wool appearing on the same sheep probably would result from the action of a series of restricting factors.

When a sheep obviously is wholly or partly pigmented, its fleece can be isolated. However, when only a few black or brown fibres arise from small pigmented areas on an otherwise white skin bearing a white fleece the economic disadvantages are greatest.

Among well-bred, selected station flocks the occurrence of obviously pigmented sheep fortunately is relatively infrequent. A series of questionnaires was sent to co-operating pastoralists who answered them after two consecutive lamb-markings. By this means it was learnt that among the progeny of some 12,000 ewes only 0·04 per cent. were described by the pastoralists as coloured or pigmented.

Information with regard to pigmented spots bearing black or brown wool fibres was secured by examining a number of sheep immediately after they had been shorn. To discover such spots extreme care must be used, because frequently they are very small and give rise to very few dark-coloured fibres.

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Their occurrence varied from zero to 74 per cent. They were not found in young Merinos of most strains, but were discovered in some particular strains. They were most common in old sheep, and were found in from 1·3 to 12·0 per cent. of all 5 and 6-year-old ewes examined. Over 5 per cent. of 3,000 ewes of all ages had such spots, and no age group was exempt. Results obtained from the examination of six ewe groups are given in Table 1.

TABLE 1.—THE OCCURRENCE OF PIGMENTED SPOTS ON THE SKIN OF EWES ACCORDING TO AGE.

Age.	Number Examined.	Average. per cent. Occurrence.
Under 1 year	700	0·5
1–2 years	416	1·2
2–3 years	500	2·2
3–4 years	342	3·5
4–5 years	348	6·3
5–6 years	275	7·2
6–7 years	216	15·7
7–8 years	213	15·0
Over 8 years	119	30·1
Mixed ages	50	2·0
Totals	3,179	5·6

The association established with advanced ages and particular strains provides guidance alike for wool buyers and sheep breeders. If buyers require white wool that is unlikely to be contaminated with pigmented fibres, they should confine their purchases to fleeces from young sheep of known strains.

Sheep breeders wishing to avoid discrimination against their clips for this reason should discard old breeding ewes and breed only from strains in which the condition does not occur.

A series of matings has been carried out which shows that "pigmentation" is a recessive character. For the purpose, a pigmented sheep was defined as one that had an obviously large area of pigmented wool. A white-wooled sheep had no obviously large patch of pigmented wool.

When pigmented sheep were mated together they gave 25 lambs. Twenty of these were pigmented, whereas five were white. Large numbers of white-wooled sheep have given white-wooled progeny. White-wooled mated with pigmented reciprocally gave 73 lambs and 30 lambs, respectively. All of these were white-wooled.

The back cross also was reciprocal, but there were few first cross (pigmented x white) females. First cross rams with pigmented ewes gave 79 lambs, 34 white and 45 pigmented. The reciprocal mating gave four lambs, three being white and one pigmented. The aggregate of the series was 83 lambs, 37 of which were white and 46 pigmented. The difference between this and 50 per cent. of each has no statistical significance.

The ratios of occurrence to non-occurrence in the first and back crosses indicate that probably only a single pair of genetic factors control the condition as defined.

This genetical analysis agrees with the conception stated earlier. It also explains why it is not an uncommon practice to use for breeding purposes, without obvious disadvantage, superior animals having areas of pigmented skin which carry pigmented wool fibres. Usually in these cases the size of the spot alone determines whether or not such animals will be excluded from breeding.

Provided the pigmented animal is mated with a suitably bred white-wooled sheep, the progeny will be white-wooled. However, if these progeny or similarly bred animals are mated, some of their offspring are bound to show pigmented fibres.

Incidental to these observations, certain hypotheses have been put forward, e.g., those already stated with regard to inhibiting and restricting factors. Another hypothesis based upon the wide range of characterization, suggests that a very large number of factor pairs are responsible for the pattern of the pigmentation. Possibly this will be found to have a normal distribution with wholly white and wholly black on the extremes of the range with all other types intermediate.

Further genetic investigation of these hypotheses would require a very large flock of sheep all pedigreed with regard to occurrence and distribution of pigment among their ancestors, and this is beyond our resources at this time.

Further Observations on the Relation of Tail Length to the Incidence of Blowfly Strike of the Breech of Merino Sheep.

*By J. H. Riches, B.Sc. (Agr.), Ph.D.**

Summary.

1. Three experiments on the effect of tail length on the incidence of crutch strike are reported.

2. All three experiments show conclusively that the longer or 4-inch tail definitely reduces susceptibility to strike of the breech area; the incidence of both true breech strike and of tail strike is reduced.

3. The effect appears to be permanent, as consistent results were obtained over a period of three years in one experiment.

4. The natural undocked tail reduces the incidence of pure breech strike to approximately the same extent as does the 4-inch tail, but is itself more frequently struck and is objectionable for other reasons.

5. There is no evidence that the longer tail reduced lambing percentages in the experimental groups.

In a previous paper (Riches, 1941) observations on the effect in two experiments of different tail-lengths on the incidence of "crutch" strike in Merino sheep were reported. It is now possible to give the more

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complete observations made in these two experiments as well as to report on observations made in a third experiment commenced in the spring of 1940.

Details of the procedure adopted in the first two experiments have already been described and need not be repeated here. In the third experiment, which like the second forms part of a larger experiment with some of the sheep treated by the Mules operation, there were only two lengths of tail employed, viz., long (i.e., 4 inches) and short.

All the animals in each of these experiments were ear-tagged at marking, and strike records have been kept for each individual. The ewes in the first experiment have lambed once, and careful observations have been made with regard to the apparent fertility of each group.

Experiment 1.

The earlier data which have been analysed in the previous report are summarized here, and details of the strikes for the period from shearing, 1940, to shearing, 1941, are now presented.

The sheep in this flock were classed and culled according to station practice in the autumn of 1940, and the numbers in the three groups were reduced thereby by approximately 30 per cent. Numbers were further reduced during 1940 by the drought which ravaged almost all eastern Australia. Consequently, at crutching in January, 1941, the numbers recorded in each group were as follows:—

Long, 74; medium, 69; short, 81.

Owing to the absence of rain and the continued dry conditions, no strike whatever was recorded after the shearing in 1940 until the end of the year. However, following the heavy rains in January, 1941, "fly-wave" conditions developed, a large number of strikes occurred, and some sheep died through lack of treatment owing to difficulty in finding them in the long grass. Details of these strikes and the losses recorded at shearing in 1941 are given in Table 1.

Table 1.—Strikes and Losses Between 20th January and 17th July, 1941.

Vital and Strike Grouping.	Grouping According to Tail Length.		
	Long.	Medium.	Short.
Number of sheep 20th January, 1941	74	69	81
Number of sheep 17th July, 1941	73	66	69
Deficiency	1	3	12
Number of sheep struck*	8	16	25
Percentage of sheep struck*	11·0	24·2	36·2
Number of strikes*	10	22	35
Strikes per 100 sheep*	13·7	33·3	50·7

* These estimates are based on the number of sheep surviving on 17th July.

It is possible that the actual mortality was not quite as high as indicated by the figures, as there is always a proportion of sheep which lose their tags, but even so the ratio of the deficiencies in the three

groups would remain approximately the same. The losses and strikes were highest in the group with the short tails.

The strikes over this period have been analysed into those involving breech only, tail and breech, and tail only. These figures are given in Table 2.

TABLE 2.—NUMBER AND LOCATION OF STRIKES OBSERVED BETWEEN 20TH JANUARY AND 17TH JULY, 1941.

Site of Strike.	Grouping According to Tail Length.		
	Long.	Medium.	Short.
Breech only	9	15	20
Breech and tail	Nil	5	8
Tail only	1	2	7

As shown in the previous report (Riches, 1941), the longer tail has produced a reduction not only in strikes affecting the tail but also in pure breech strikes.

Table 3 gives a summary of the strike incidence in the three groups in the experiment from its inception in 1938 to shearing in 1941.

TABLE 3.—STRIKES PER 100 SHEEP IN THE THREE GROUPS AT INTERVALS SINCE MARKING IN NOVEMBER, 1938.

Interval.	Grouping According to Tail Length.		
	Long.	Medium.	Short.
From marking November, 1938, to crutching 23rd May, 1939.	32·2	55·9	61·0
From 23rd May, 1939, to shearing, 23rd August, 1939 ..	5·0	17·0	19·0
From 23rd August, 1939, to classing, 20th March, 1940 ..	2·0	0·0	2·0
From 20th March, 1940, to shearing, 17th July, 1940 ..	1·0	7·0	8·0
From 17th July, 1940, to crutching, 20th January, 1941 ..	0·0	0·0	0·0
From 20th January, 1941, to shearing, 17th July, 1941 ..	13·7	33·3	50·7

It is obvious from these figures that the effect of the longer tail in reducing the incidence of strike has been permanent.

Experiment 2.

As stated previously, this experiment forms part of a larger one involving also the Mules operation, which will be reported on fully elsewhere (Riches and Johnstone, 1942). It was commenced in 1939, and four tail lengths were adopted, viz., medium, long, and short, as in Experiment 1, as well as a group with natural undocked tails.

Owing to the drought conditions prevailing during the summer of 1939 and the whole of 1940, no strikes were recorded until after the drought broke in January, 1941. When the sheep were crutched on 29th January there was a fairly high incidence of strike, and "fly-wave" conditions prevailed in the succeeding months until the sheep were shorn on 19th May.

Owing to the difficulty in finding struck sheep in the long grass there was some mortality from strike and, as in the previous experiment, this is shown in the figures recorded at crutching and shearing. Table 4 gives details of the strike incidence at crutching and between crutching and shearing, also of apparent losses in the different groups over the same period.

TABLE 4.—APPARENT LOSSES BETWEEN CRUTCHING ON 20TH JANUARY AND SHEARING ON 19TH JULY, 1941, AND STRIKE INCIDENCE OVER THE SAME PERIOD.

Vital and Strike Grouping.	Grouping According to Tail Length.			
	Long.	Medium.	Short.	Undocked.
Number of sheep 20th January, 1941 ..	166	161	160	157
Number of strikes 20th January, 1941 ..	14	45	57	19
Strikes per 100 sheep	8·4	27·9	35·0	12·1
Number of sheep 19th July, 1941	157	138	135	155
Deficiency	9	23	25	2
Number of sheep struck between 20th January, 1941 and 19th July, 1941*	24	57	81	46
Percentage of sheep struck*	15·3	41·3	60·0	29·7
Number of strikes*	39	84	122	64
Strikes per 100 sheep*	24·8	60·9	90·4	41·3

* These estimates are based on the number of sheep surviving on 19th July.

These figures were also analysed to show strikes involving the breech only, the tail only, as well as tail and breech together. These figures are given in Table 5.

TABLE 5.—NUMBER AND LOCATION OF STRIKES OBSERVED BETWEEN 20TH JANUARY AND 19TH JULY, 1941.

Location of Strike.	Grouping According to Tail Length.			
	Long.	Medium.	Short.	Undocked.
Breech only	30	69	86	27
Breech and tail	9	12	29	25
Tail only	Nil	3	7	12

Study of these two tables shows that the results obtained in Experiment 1 are confirmed, as well as the conclusions which may be stated as follows:—

(*a*) That the long, four-inch tail has given very considerable reduction of susceptibility to strike as compared with the orthodox medium tail.

(*b*) That a very short tail increases susceptibility to strike to an appreciable extent.

(*c*) That the reduced incidence of strike in the longer tailed group is not due to a reduction in tail strikes only, but also to reduction in the number of pure breech strikes.

A study of the figures for the undocked group as given in Table 4, shows the incidence of strike in this group to be considerably higher than that in the "long-tailed" group, but appreciably lower than in the

group with medium tails. Table 5 shows that the incidence of pure breech strike is almost identical in the groups with "long" and undocked tails respectively, but the incidence of pure tail strike and of mixed strike involving the tail is much higher in the undocked group than in the "long-tailed" group. A considerable number of strikes were noted about half-way down the undocked tail at a point which would be absent from the "long" tail. It thus appears that the natural tail exerts the same protective influence agains breech strike as does the "long" tail, but that it is much more liable to tail strike. From the fact that the incidence of pure breech strike is almost identical in these two groups, it would appear that there is no further protection to be gained by leaving a tail longer than 4 inches.

Experiment 3.

This experiment was commenced in October, 1940, and also forms part of a larger experiment (Riches and Johnstone, 1942). Two tail lengths only were tested, viz., "long" (i.e., 4 inches) and "short" (i.e. about 1 inch). These short tails are somewhat longer than those in Experiments 1 and 2, but are still appreciably shorter than the medium tail. Wethers as well as ewes were included in this experiment, and results for the two sexes will be given separately.

No strike was recorded between marking in October and crutching in January, but subsequent to crutching a fair amount of fly activity was in evidence, and this continued until shearing in May.

In April a number of individual sheep in this experiment showed signs of becoming daggy, and it was hoped that information might be obtained on the question as to whether the "long" tail would be more subject to dagginess than the orthodox medium tail. Unfortunately, however, the feed dried off rapidly and the dagginess did not increase. On 24th April all those requiring it were dagged, and note was made of the individuals treated. It was found that no ewes needed dagging, and of the wethers 6·5 per cent. in the "long" tailed and 10 per cent. in the "short" tailed groups required attention. The numbers are, unfortunately, insufficient to provide adequate evidence either way; all that can be said is that there is nothing to indicate that the longer tail is at a disadvantage compared with the short tail. The incidence of breech strike for both sexes for the period is given in Table 6.

Table 6.—Incidence of Breech Strike in Ewe and Wether Weaners Between 1st January and 23rd May, 1941.

Vital and Strike Grouping.	Grouping According to Sex and Tail Length.			
	Ewes.		Wethers.	
	Long.	Short.	Long.	Short.
Number of sheep at 23rd May, 1941 ..	104	105	102	97
Number of sheep struck	19	46	2	3
Percentage of sheep struck	18·3	43·8	2·0	3·1
Number of strikes	20	59	2	3
Strikes per 100 sheep	19·2	56·2	2·0	3·1

It will be seen that the strike incidence in the wethers has been very light, and numbers are not sufficient on which to form an opinion of the value of the longer tail in this class of sheep. In the ewes, however, the value of the longer tail has again been demonstrated. When the strike figures were analysed for location, it was found that only one pure tail strike and one mixed tail and breach strike had occurred in each group. The reduction brought about by the longer tail had, therefore, in this instance, been entirely in pure breech strikes.

Lambing Percentages.

One criticism which has been levelled against the adoption of the longer tail is that it will interfere with mating, and thereby reduce lambing percentages. The 1938 ewes were mated as maidens in 1940, but owing to drought conditions, very few lambs survived, and it was impossible to obtain any figures on lambing percentages. These ewes were again mated in April, 1941, and have since lambed. Shortly after the completion of lambing the lambs were marked, and the ewes were examined carefully to determine those which were suckling lambs, those which had given birth to lambs but whose lambs were either born dead or had died shortly after birth, and those which had not produced a lamb at all. Table 7 gives details of the distribution of these three classes.

Table 7.—Record of Maternity of the Groups.

	Grouping According to Tail Length.					
	Long.		Medium.		Short.	
	Nos	Per Cent.	No.	Per Cent.	No.	Per Cent.
Ewes suckling lambs ..	50	72·5	47	71·2	49	79·0
Ewes which lost lambs ..	9	13·0	6	9·1	6	9·7
Completely dry ewes ..	10	14·5	13	19·7	7	11·3
Total number of ewes ..	69	..	66	..	62	..

At first glance, from the figures for ewes suckling lambs, it would appear that the short-tailed group is definitely superior. If the figures for all ewes which delivered lambs, or conversely those for completely dry ewes, are compared, it appears that the "long" group is superior to the "medium" group by 5·2 per cent., whereas the "short" group is superior to the "long" by a further 3·2 per cent. As the medium tails are not likely to have a more detrimental effect on conception and gestation than either long or short tails, we must conclude that the differences noted are due to chance, and there is no evidence that any particular length of tail has had an effect on lambing percentages.

The ewes in Experiment 2 were not mated in 1941, but will be observed carefully when they lamb in 1942.

Discussion.

Careful records of individual wool production were kept for all three experiments, and these have been analysed. In all three experiments there were slight differences in favour of the long tail, but they were so small that they could not be regarded as significant.

When the figures for all three of the experiments reported are considered, it becomes obvious that very short tails definitely increase susceptibility to strike and, conversely, that a tail about 4 inches long decreases susceptibility. Further, the proportionate reduction in strike is greater between the "medium" and "long" than it is between the "short" and "medium" tails. It is a little difficult to understand why this should be so, but in view of the fact that it has occurred reasonably consistently throughout the experiments it must be accepted.

The results obtained with the undocked tail show that compared with the "long" tail it has nothing to recommend it. It apparently reduced pure breech strike to the same extent, but was more subject to tail strikes. Further, the majority of the undocked tails became very dirty at the extremity and also soiled the hocks rather badly. In some cases, indeed, the wool was rubbed off the hocks completely and sores developed.

The "long" or 4-inch tail has been criticized by a number of graziers on two points, viz.:—

(*a*) That the longer tail will tend to become very much more daggy than the orthodox "medium" tail in lush seasons or on country where succulent pastures are the rule. Unfortunately, no evidence for or against this contention has been obtainable. The seasons under review in these experiments have not been of such a nature as to engender scouring and the production of dags.

(*b*) That the longer tail will tend to interfere with mating and thereby reduce lambing percentages. The evidence so far obtained is not conclusive, and probably the most that can be said at present is that the criticism is not supported.

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The Rugging of Sheep in Australia.

By I. W. Montgomery, B.V.Sc., and C. C. Blumer, B.V.Sc.***

1. Introduction.

Many articles which have appeared in the agricultural press have spoken favourably of rugging. The benefits which were said to result from rugging sheep included increased body weight and wool production, improvement in wool quality, higher lambing percentages, increased growth rate of lambs from rugged ewes, and better general health.

Controlled sheep rugging trials in Australia, however (Blumer and Cotsell, 1938; Montgomery, 1938-39; Peirce, 1938; Levy, 1939; Elliott, 1939; Thomas and Throssel, 1939), have shown no increase in body weight or wool production among rugged sheep. The only advantage for which there is adequate proof is that rugging protects the fleece from dust and burrs. The observation by Duncan (1939) in New Zealand that rugging tends to prevent fleece-rot suggests that it would reduce the incidence of that form of blow-fly strike known as body strike, which is usually associated with fleece rot. There is no evidence that it has any other effect on the incidence of fly strike, and Keast (1937) has pointed out that strikes about the tail and breech may extend widely under the edge of the rug.

There appear to be no serious objections to rugging apart from the cost of the rugs, their relatively short life, and the increased labour required for their care and adjustment.

It appeared that further investigations were required concerning: (1) the effect on food utilization of rugging ewes in cold climates as reflected in their body weight and ability to bear and rear lambs, and (2) the effect of rugging on general health and thus, indirectly, on gastro-intestinal parasitism.

Sound judgment of the economics of rugging Merino sheep under Australian conditions could only be made after due consideration had been given to these indirect as well as the direct effects.

2. Experimental Procedure.

Trials with ewes and with weaners were commenced at "Saumarez," near Armidale, in November, 1938.

Three groups, each of 75 aged ewes, were selected at shearing. The ewes were selected in lots of three for uniformity of body weight, of total greasy wool weight, and of fleece characteristics. The individuals of each lot of three were then allotted at random to form the groups of 75 ewes. Group 1 was rugged from shearing in November, 1938, till its lambs were weaned in March, 1940. Group 2 was rugged from May, 1939, until shearing in October, 1939, i.e., during the winter and spring. Group 3 was not rugged. The ewes lambed in late September and early October, 1939. They were shorn on 31st October and the lambs were weaned in March, 1940.

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** District Veterinary Officer of the New South Wales Department of Agriculture, stationed at Armidale.

For the trial with weaners, a rugged group and a control group were used. Each group contained 50 ewe weaners and 50 wether weaners which had been selected and allocated to the respective groups as was done with the aged ewes. This trial commenced early in May, 1939, and ended at shearing in November, 1939.

The sheep in both trials were all weighed, and wool samples as well as faeces samples were collected from selected animals at monthly intervals. The lambs born to the ewes were weighed at birth and at intervals thereafter. The quantity and quality of each sheep's wool were carefully examined and recorded at shearing, actual yields were calculated on selected samples, and the wool was officially appraised under the Wool Appraisal Scheme. The costs involved by rugging were kept. Fibre diameter and staple length were measured on selected sheep at intervals throughout the trials.

3. Results.

The mass of data derived from these observations is not published in full owing to restrictions on space imposed by the war. Under present circumstances it must suffice to give the final results of the two trials briefly, as follows:—

(*a*) *Aged Ewes.*

1. The mean body weights of the three groups remained almost identical throughout the trial.

2. No significant differences were detected in the degree of internal parasitism.

3. There was no material difference in the total production of greasy wool, but since heavier skirting was necessary in the group which was rugged throughout the year, the amount of fleece wool, as distinct from total wool, was lower in this group.

4. The clean scoured fleece weight of the group rugged throughout the year was slightly lower than that of the other two groups, although its percentage clean scoured yield was higher.

5. Of the fleeces from the group rugged the whole year, 31 per cent. were in the top commercial line, whereas only 8·3 per cent. and 2·8 per cent. respectively were so classed from the other two groups.

6. The proportion of fleeces in the low spinning quality range was significantly higher among the unrugged ewes compared with the 1938 and 1939 shearings, whereas in the rugged groups no such alteration occurred.

7. When the staples were divided into four zones corresponding roughly to the four seasons of the year, it was found that all groups showed a definite decrease in fibre diameter from the tip zone to the base, i.e., from summer to winter. This decrease was less pronounced in the group rugged for the whole year than in the other two groups. There was no significant difference between the groups as regards mean fibre diameter taken over the whole length of the wool fibres. Rugging thus appeared to be associated with a slightly more uniform growth of fibre, though the difference was not great.

8. The group rugged throughout the year grew a staple about 0·25 cm. longer than the others, but this difference is thought to be of no practical importance. No appreciable differences were found as regards soundness or colour of the wool.

9. Differences between the total values of wool from the three groups under the Wool Appraisal Scheme were negligible. Wool from the group rugged for the whole period realized £28 18s., that from the group rugged during winter and spring £30 1s. 2d., and that from the unrugged group £28 19s. 3d. These figures have been adjusted to a total for 72 sheep in each group. Rugging costs were estimated at 1s. 3½d. per head, the cost of rugging 72 sheep thus being £4 13s.

10. The ewes lambed in September, 1939, and there were no significant differences between the groups in lambing percentage, the mean birth weights of lambs, nor in the growth rate of the lambs between birth and weaning.

11. Thus, in a district which is representative of the colder, highland, sheep-raising areas of N.S.W., the rugging of aged Merino ewes showed a financial loss when rugging costs were offset against wool returns. There was no compensating gain in general well-being of the rugged ewes or in their lamb production.

(b) Weaners.

1. No appreciable advantage resulted from rugging as regards body weight, quantity or quality of wool produced, or the degree of internal parasitism.

2. When wool valuations were adjusted to 100 weaners in each group, the wool of the rugged group was valued at £36 18s. 7d. and that of the unrugged group at £36 6s. 3d.

3. Rugging costs were estimated at 1s. 0½d. per head, or £5 4s. 2d. per 100 weaners. It is clear, therefore, that under these conditions rugging involved an appreciable financial loss.

4. Acknowledgments.

A trial such as this, which involved over 600 sheep, over 10,000 figure records, and monthly examination of the experimental sheep, demanded the co-operation of a large team of workers and the whole-hearted co-operation of the owners and staff of the property "Saumarez," on which it was conducted.

Officers of the Council for Scientific and Industrial Research, the N.S.W. Department of Agriculture, and the Wool Manager and his assistants at the New England North and North-west Producers Co. Ltd. ("Nenco") gave essential co-operation in this trial.

All this assistance is gratefully acknowledged by the authors.

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Yellow Dwarf of Tobacco in Australia.

III. Occurrence and Effect of Agronomic Practices.

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Summary.

1. Yellow dwarf, a virus disease of tobacco, occurs in Southern Queensland, New South Wales, Victoria, and South Australia. In Victoria and in the Tamworth district of New South Wales it is economically important every year.

2. The time during which infection most commonly occurred varied with seasonal conditions. Greatest loss resulted if the main infective period followed soon after the main transplanting period.

3. During a period of five years, observations were made on the effect of a number of factors, including variety and source of seed, seedbed and seedling treatment, and soils and fertilizers, on incidence of the disease, but none appeared to be of importance. In some seasons there were comparatively few diseased plants in late-planted crops.

4. The greatest differences in percentage infection were between different districts in any one season, and between different seasons.

1. Introduction.

In recent years yellow dwarf (2, 3) has seriously affected the tobacco-growing industry in Victoria and some districts of New South Wales. It has reduced yield and value and in some seasons was so widespread that some crops were worthless. During successive years, the yield per acre has been low, confidence in the industry being thereby undermined and other crops substituted for tobacco.

From 1936 to 1940, field surveys were made to determine the distribution of the disease and the effect on its occurrence of various cultural practices and treatments. Where possible, the data were analysed statistically and much of the information was summarized for inclusion in this paper.

2. Occurrence.

(a) Districts.

Although tobacco is grown in all States of the Commonwealth, yellow dwarf has not been reported from Western Australia, Tasmania, and the northern and coastal areas of Queensland. During the last five years very few diseased plants have been found in South Australia or in the Texas–Yelarbon district on the Queensland–New South Wales border, but in other parts of New South Wales and in Victoria, the disease has been widespread, especially during the 1937-38, 1938-39, and 1939-40 seasons.

At Ashford, N.S.W., about 60 miles south of Texas, up to 15 per cent. infection occurred. Further south, at Tamworth, almost all plants in some crops were diseased in some of the years under review—the estimated percentage infection for the district varying from 8 to 50. In the southern part of the State the loss due to the disease was one

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of the important factors that eliminated Tumut as a tobacco-growing area. During the last eleven years tobacco crops grown in a number of isolated districts south of Tamworth were observed to be heavily infected by yellow dwarf.

Over 90 per cent. of the tobacco crop in Victoria is produced in the Ovens River valley in the north-east of the State, and the remainder at Shepparton, Nathalia, and Gunbower. Tobacco is sometimes grown in North Gippsland and at Pomonal. A high percentage of diseased plants occurs each year in the Shepparton, Nathalia, and Gunbower areas. At Pomonal more than 90 per cent. of the plants in some crops are affected in some seasons, but in other seasons the loss is small. Only a few diseased plants are found in the crops grown in the Gippsland area. In the Ovens Valley, 10 to 20 per cent. of dwarfed plants in the crop are expected by growers, but in some years the proportion of diseased plants is so high that some crops are plowed under.

The more important of the tobacco-growing districts in which yellow dwarf occurs and some weather data for the area as a whole are shown in Fig. 1. In Fig. 2 is given the estimated percentage of infected plants in seasons and districts for which there are sufficient data. It will be observed that high percentages occurred in 1938-39. During the two following seasons, the percentage infection probably increased at Texas–Yelarbon, Ashford, and Tamworth, remained substantially the same in the Shepparton–Nathalia–Gunbower areas, and decreased by about 40 per cent. in the Ovens Valley. Information concerning each district is not available for all seasons.

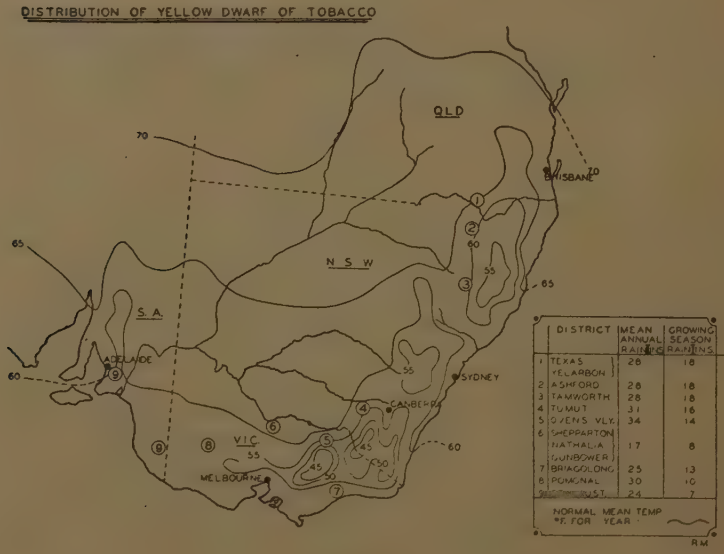

FIG. 1.

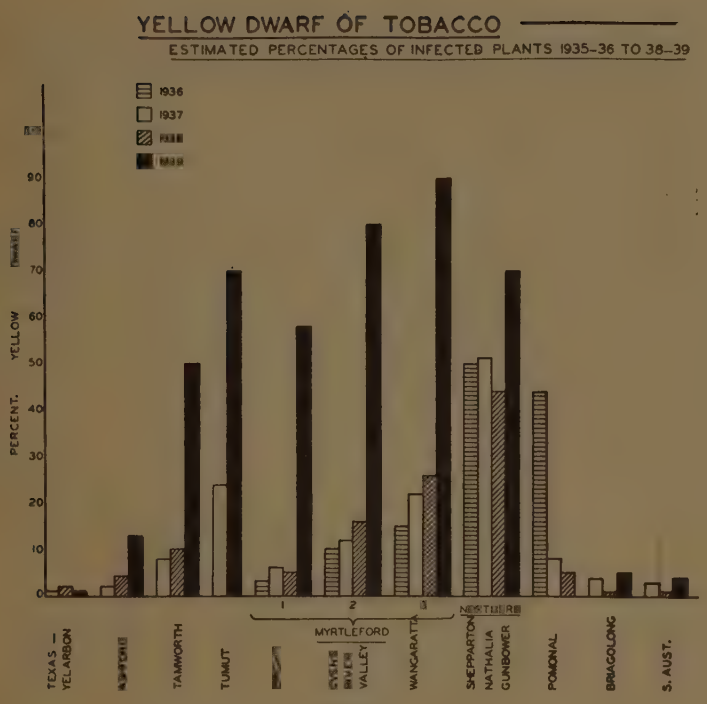

Fig. 2.

(b) Weather conditions.

An examination of maximum and minimum temperatures and rainfall in the tobacco-growing areas did not reveal any outstanding differences that might help to account for relative freedom from yellow dwarf in some areas and a high proportion in others. The climates of North Queensland and Western Australia, where the disease does not occur are, however, quite different from that of the affected area. North Queensland is tropical, tobacco being grown during the short, wet season (4). The climate of the tobacco areas of Western Australia is somewhat like that of Victoria, but the winter rainfall is greater, the spring–summer period much drier, and the atmospheric humidity higher.

Topographically the Ovens Valley, Tumut, and Tamworth areas are somewhat alike—rather narrow valleys that widen into comparatively level country. The Ovens Valley district is about 50 miles long and in some places several miles wide. Bright, situated at the southern end, is almost surrounded by mountainous country; the rainfall is higher and the temperature lower than in the plain around Wangaratta where the percentage of diseased plants is almost invariably much higher. The districts of Shepparton, Nathalia, and Gunbower, where a high percentage of diseased plants is expected every year, are irrigation areas in an extensive plain.

The mean annual temperature of the most northerly area in which yellow dwarf occurs is about 68°F. and that of the most southerly area 55°F. (7). Rainfall is fairly evenly distributed throughout the year and in tobacco-growing areas is supplemented by artificial watering. Wheat is a crop of major importance in this climatic zone. In the pasture map of Australia (5) most of the area is described as open grassland—southern (*Danthonia,* &c.).

Weather conditions prevailing during the summer may modify the degree of dwarfing. If the season is dry and hot, infected plants may flower at a height of 12 to 18 inches. If there are good summer rains some plants will grow comparatively well and produce marketable leaf; it is, however, noteworthy that this response is not obtained by artificial watering in dry seasons. A somewhat similar result was obtained on a very sandy area following heavy topdressing with organic manure before transplanting. The percentage infection in the topdressed area was higher but most of the diseased plants produced marketable leaf, whereas in the untreated area the leaf was almost worthless.

Temperature and rainfall data for the infection period of the worst season on record in Victoria for yellow dwarf are given in Table 1.

TABLE 1.—TEMPERATURE AND RAINFALL, OVENS VALLEY, VICTORIA, 1938. WEEKLY MEAN MAXIMUM AND MINIMUM TEMPERATURE FOR INFECTION PERIOD OF YELLOW DWARF OF TOBACCO.

Week Ending—		Mean Maximum.	Mean Minimum.	Rainfall.		
				Bright.	Myrtleford.	Wangaratta.
		deg. C.	deg. C.	in.	in.	in.
October	30 ..	25·3	8·8	8	5	0
November	6 ..	26·3	10·8	86	34	12
	13 ..	27·5	8·2	21	4	0
	20 ..	33·7	10·2	0	0	0
	27 ..	28·2	11·6	0	7	2
December	4 ..	25·5	7·8	8	6	0
	11 ..	26·6	9·0	17	12	0
	18 ..	28·8	8·5	3	7	0
	25 ..	32·8	11·7	0	0	3

The temperatures given are derived from thermohygrograph records made at Ovens, Myrtleford, Whorouly, Markwood, and Wangaratta.

In the northern districts of Victoria, the minimum temperatures, after the first three weeks, were 1°–3°C. higher.

(*c*) *Symptoms.*

In all districts, infection by the yellow dwarf virus occurs most commonly during the latter half of November. In some seasons it may be earlier or later—it was late in 1939-40—but the variation is seasonal and not from district to district. Usually the proportions of diseased plants in different crops transplanted in a district at the same time are about equal. All crops planted before the main infective period are usually equally affected. In Southern Queensland a few infected plants occur in most crops, whereas there are many in all crops in Northern Victoria. Usually, comparatively few diseased plants occur in crops transplanted late in December.

Dry, hot conditions during and for some weeks after the transplanting period appear to favour the spread of the disease and the early appearance of symptoms. The first diseased plants are usually observed in the field two or more weeks after infection, but in field experiments and insect transmission tests, the minimum period was ten days. Small rapidly-growing plants appear to be very susceptible to the disease. Symptoms may be more clearly defined after showery weather, possibly because the more rapid growth of healthy plants provides a greater contrast with diseased plants.

Infection may take place at any stage in growth, but the loss is greatest if plants are attacked soon after transplanting. Sometimes the maximum height of almost every plant in the field is 15 inches. A more common feature of the disease is unevenness in the crop—large and apparently healthy plants occurring at random in a field where most of the plants are more or less stunted. According to Penman (6), analyses of yellow dwarf plants reflect disturbances in mineral nutrition, one being an abnormal ratio of lime to potash in the leaves. At, or after the flowering stage, it is often difficult to determine accurately the percentage infection in the crops. Such yellowing as occurs at this time may be due to the disease or be associated with maturation of the leaf. However, typical symptoms occur in leaves that develop from axillary buds.

3. Effect of Agronomic Practices on Occurrence.

Observations over a five-year period were made in fields in different districts to determine if agronomic practices affected the occurrence of yellow dwarf. Source of seed, seedling production, varieties, soils, fertilizer treatment, time of planting, and cultural methods were investigated. Observations on occurrence of the disease in field plots of the various State Departments of Agriculture were of particular value. Wherever possible, treatment results were examined by statistical methods.

(*a*) *Seed.*

Seed was suspected by growers as a carrier of the disease, consequently care was taken by them to collect seed from apparently healthy plants. As disease-free crops rarely, if ever, occurred in the affected areas, and as plants infected at a late stage in growth were not readily detectable, plant selection by the grower was not likely to be effective. In these investigations it was found that crops grown from seed produced by plants in isolated fields almost free of disease were just as much affected as others from seed produced in fields with higher percentages of diseased plants. Seed from Western Australia, where yellow dwarf does not occur, did not give any better results than seed from affected areas. In one test in which seed from (*a*) Western Australia and (*b*) a diseased crop was used, the seedlings were grown in an isolated area several hundred miles from tobacco-growing districts and also in benzol beds near the field into which they were subsequently transplanted. Seedlings from each lot of seed and from each seedbed were transplanted to the same field; all were equally affected by yellow dwarf. Similar results with seed from various sources were observed in fields in several districts.

Seed from different sources was used in a number of experimental field plots. Data for one such test conducted in Briagolong, Vic., are summarized in Table 2.

TABLE 2.—OCCURRENCE OF YELLOW DWARF IN PLANTS GROWN IN 1938–39 FROM SEED PRODUCED BY INFECTED AND APPARENTLY HEALTHY PLANTS.

Variety.	Source of Seed.	Total Plants.	Yellow Dwarf.
			per cent.
Dungowan ..	Yellow dwarf plants—Ovens Valley, Victoria, 1937–38	160	5
Dungowan ..	Apparently healthy plants—Ovens Valley, Victoria, 1937–38	242	8
Hickory Pryor ..	Yellow dwarf plants—Ovens Valley, Victoria, 1937–38	71	1·4
Hickory Pryor ..	Apparently healthy plants	394	6
Mixed	Australia and overseas countries. Plants of each in proportion of 3 : 1	8,167	5·3

In another test at Nathalia, Vic., an area of 4 acres was planted with two varieties, the seedlings being from seed of apparently healthy and diseased plants of each variety. Seedlings from each of the four lots of seed were transplanted at weekly intervals beginning 2nd November and ending on 16th November, 1940. There were no significant differences in the percentages of diseased plants. The results are summarised in Table 3.

TABLE 3.—OCCURRENCE OF YELLOW DWARF IN PLANTS GROWN IN 1940-41 FROM SEED PRODUCED BY (*a*) INFECTED AND (*b*) APPARENTLY HEALTHY PLANTS.

Date of Transplanting.	Variety.	Total Plants.	Yellow Dwarf.
			per cent.
2nd November, 1940 ..	Hickory Pryor (*b*) ..	1,837	87
	„ „ (*a*) ..	1,905	91
	Dungowan (*b*)	2,320	95
	„ (*a*)	1,412	98
9th November, 1940 ..	Dungowan (*a*)	1,683	96
	„ (*b*)	1,778	94
	Hickory Pryor (*a*) ..	2,001	92
	„ „ (*b*) ..	1,651	93
16th November, 1940 ..	Hickory Pryor (*b*) ..	1,963	91
	„ „ (*a*) ..	2,232	91
	Dungowan (*b*)	1,500	95
	„ (*a*)	2,090	93

(*a*) Seed from yellow dwarf plants.
(*b*) Seed from apparently healthy plants.

During the three years 1936, '37, and '38, seed from varieties grown in Australia and a large number of varieties and strains of imported seed were sown at Canberra and the seedlings transplanted in

randomized plots at Briagolong, Vic. The data, summarized in Table 4, indicate that source of seed does not influence the amount of disease in the field.

TABLE 4.—OCCURRENCE OF YELLOW DWARF IN TOBACCO PLANTS GROWN FROM SEED PRODUCED (*a*) IN AUSTRALIA AND (*b*) IN OTHER COUNTRIES.

Origin of Seed.	Year Grown.	Number of Countries Represented.	Varieties and Strains.		Total Plants.	Yellow Dwarf.
			Total.	Number with Yellow Dwarf.		
						per cent.
Outside Australia* ..	1936–37 ..	11	274	54	2,663	3·7
	1937–38 ..	8	276	25	4,579	·6
	1938–39 ..	10	278	72	2,339	4·7
Australia	1936–37 ..	1	15	4	171	3·5
	1937–38 ..	1	224	11	2,198	·5
	1938–39 ..	1	483	200	5,325	5·4

* The seed was produced in 14 countries, all continents included.

(*b*) *Varieties.*

At Duntroon, A.C.T., in 1936, 13 varieties from seed produced in three continents were planted in one half-acre plot. There were no significant varietal differences in the proportions of diseased plants.

In other variety tests a few species of *Nicotiana* other than *tabacum* were included. Yellow dwarf was observed in *N. rustica, N. chinensis, N. purpurea,* and *N. trigonophylla.* Under greenhouse conditions it was also transmitted by grafting to *N. glutinosa* and *N. glauca,* the latter being symptomless under the conditions of the experiment.

Variety trials conducted by the Victorian Department of Agriculture at several centres in the years 1936-37, 1937-38, and 1938-39 were examined for the incidence of yellow dwarf. At Myrtleford in 1936-37, one trial consisted of five randomized blocks of the varieties Hickory Pryor, Dungowan Selection, Conqueror, Adcock, and Yellow Pryor. The data from this experiment were analysed statistically, and it was found that the varietal differences in numbers of infected plants were not significant. There were trials with a number of other varieties, the counts from which could not be treated by the analysis of variance. It was apparent, however, that the amount of disease was approximately the same in all these as in the other varieties that were included in the statistical analysis. There was an average of 9 per cent. infection over the whole experimental area, and the general conclusion drawn from this set of trials was that varietal effects on the incidence of yellow dwarf could not be detected.

In 1937-38, observations were made on variety trials at Gunbower, Nathalia, Myrtleford, Markwood, and in two fields at Pomonal. At Nathalia, where there were 11 varieties in five randomized blocks, the percentages of infection showed significant varietal differences. At the other places the variety data were not suitable for statistical analysis. However, the 4 varieties, Conqueror, Dungowan Selection, Yellow

Pryor, and White Stem Orinoco were all included in the trials at Myrtleford, Markwood, Gunbower, and Nathalia, and the percentages infection from these were tested by the analysis of variance. Differences between districts were very much greater than any of the differences between varieties. Nevertheless, there were significant varietal differences, Dungowan and White Stem Orinoco having higher percentages of infection than Conqueror and Yellow Pryor. The infection at Gunbower was generally greater than elsewhere, being about 40 per cent. Though the same varieties were not grown in all places, there was sufficient overlapping for it to be apparent that at Pomonal the degree of infection was approximately the same order as at Myrtleford and Nathalia, that is, about 10 per cent.

At Markwood, some varieties failed to grow satisfactorily and the amount of infection was very variable. The average of infection was about 25 per cent. in the fertilizer trial and 14 per cent. in the variety trial. In the variety test the percentage infection varied from 7 to 38. Since the variety trial was not replicated, each variety being in a solid block in the field, some of the apparent varietal differences may have been due to positional effects.

In 1938-39, the variety trial data from Nathalia could not be analysed statistically, since there was no true replication, and consequently no error term could be calculated for testing the significance of differences. Percentages of infected plants were calculated, based on the final stand, which averaged 234 plants per variety. The following results were obtained:—

Percentage of Infected Plants.

Dungowan Selection.	Hickory Pryor.	Cash.	Kelly.	Conqueror.	Gold Dollar.	White Stem Orinoco.	White Mammoth.	Kentucky Yellow.	Dungowan
76	76	78	71	75	68	62	68	63	61

Although the above table seems to indicate varietal differences, these may be due to positions in the field. The varieties were grown in the field in the order in which they are listed above. There is, therefore, a suggestion of a gradient from one end of the field to the other, and this might account for the apparent varietal differences. In the absence of replication, it is not possible to distinguish between these two effects. It is to be noted, also, that in the 1937-38 trials, the variety White Stem Orinoco had a significantly higher infection than Conqueror, and in this trial it was much lower.

At Myrtleford in 1938-39, 11 different varieties and 10 strains of 3 other varieties were planted in one block, there being two rows, each of approximately 100 plants, of each variety or strain. Transplanting was done on 18th October and observations were made on 2nd February, but no varietal differences were apparent. The percentage of infected plants varied from 95 to 100, the average height of all plants, including inflorescences, being about 2½ feet.

From these trials conducted over a period of three years, it can be concluded generally, that some varietal differences existed, but these were peculiar to localities and seasons. The most conspicuous differences

in percentage infection were between different places in any one season, and between different seasons. Variety trials in other States and in different seasons were also examined for the incidence of yellow dwarf, but there was no indication that any of the varieties used was inherently less susceptible to the disease than other varieties.

(*c*) *Seedbeds and Seedlings.*

The seedlings used in each variety test were usually grown in one lot of seedbeds, therefore the almost uniform percentage of disease might have been assumed to have been due to random infection in the seedbed. However, it was shown repeatedly that the percentage of infected plants was not influenced by the origin of the seedlings. In these experiments, seedlings grown in one district were transplanted in several districts, the percentage infection varying according to the general average for the district in which they were transplanted. A similar result was obtained if lots of the same seed were sown in various districts and the seedlings transplanted in the district where they were grown, or in one field in a district. In a typical instance, 61 per cent. of the plants in a field at Nathalia were infected with yellow dwarf, whereas at Myrtleford only 11 per cent. from the same seedbed, transplanted at the same time, were affected. In most years, a few yellow dwarf plants were observed in seedbeds late in the season, but the highest observed infection in them was only 3 per cent., whereas in adjoining fields more than 30 per cent. of the plants were diseased. It does not appear, therefore, that infection in the seedbed is of importance.

In several tests the seedlings were from seedbeds treated with formalin or steam and from untreated beds. Another trial combined fertilizer and seedbed soil treatment, using the variety Orinoco Pride. The soil treatments were steam, no treatment, aretan*, and formalin, and the fertilizers, no manure, 300, 500, and 700 lb. of superphosphate per acre. There was no true replication, there being only one row of each, except in the steam-treated classes, for which there were sometimes two and sometimes three rows. The different factor combinations were allocated at random to the rows, and consequently it was possible to make an analysis of variance on percentage infection, using the interaction between seedbed soil treatment and fertilizer as the error term. One subclass, the P.7 steam-treated, was missing altogether and a value for this was estimated by Yates' method (8). The analysis of variance showed no significant effects due either to the fertilizers or to the seedbed soil treatments.

At Shepparton in 1937-38, spraying trials for the control of downy mildew in seedbeds were conducted with the variety Dungowan Selection. The treatments included benzol vapour, no treatment, and the following sprays:—Bouisol, copper emulsion, shirlan, and collodial copper. There were three series, one sprayed weekly, one sprayed twice per week, and another sprayed three times per week. Seedlings from each of the treatments were transplanted in the field in the form of a randomized block experiment, with four blocks and four treatments. Later, the numbers of yellow dwarf plants in each treatment were counted, and an analysis of variance of percentages of infection was made for each experiment. There were no significant treatment effects. It was not

* A proprietary water-soluble organo-mercury compound.

considered necessary to transform the data before analysis, because there was a fairly high percentage infection (about 20 per cent.). There were obvious differences in the degree of infection in the three series, and as the treatment with benzol and the control occurred in all three, these differences apparently represent a positional effect. Although there were no significant effects in any one of the three experiments, infection of the benzol-treated plants was rather lower than the controls in each of the three series, and this may, therefore, represent a genuine effect. It is to be noted, however, that there was a very large number of replacements among the plants treated with benzol, and this is probably the cause of the lower infection in these plots; it is likely that the replacements missed the possibility of infection to which the others were subjected.

Observations were also made on the effect of seedbed site and soil on subsequent incidence of the disease in transplants. None of the observations, made over the five-year period, suggested that the percentage infection of yellow dwarf in the field was affected by the conditions under which seedlings were produced.

(*d*) *Soils.*

Yellow dwarf occurs on all soil types from deep infertile sands to heavy clay-loams. Under adverse conditions of soil and weather, diseased plants grow very slowly. After good summer rains, further growth may occur in leaves already nearing maturity, or axillary buds may develop, but the leaves produced are seldom of commercial value. On rich soils of good texture or on soils in which large quantities of humus have been incorporated, diseased plants sometimes continue to grow and eventually produce leaves of commercial value. In poor soils and under unfavourable soil conditions, stunted and yellowed plants not infected with yellow dwarf were often observed, but under improved conditions such plants usually recovered and grew in a normal manner.

The percentage infection of yellow dwarf was observed in comparable crops grown on different soil types, on old tobacco land, virgin land, or land under rotation crops for some years. These observations, made in various districts, did not indicate that the proportion of diseased plants was affected by any of these soil treatments.

(*e*) *Fertilizer treatment.*

Observations were made on the occurrence of yellow dwarf in fertilizer plots of the Departments of Agriculture in Victoria, New South Wales, and South Australia. When possible, the percentages of infection in the plots were analysed statistically.

At Myrtleford, Victoria, in 1936-37, the variety Hickory Pryor was used in fertilizer experiments. Initially there were 50 plants per plot, the seedlings for which had been taken from benzol beds. There were three randomized block experiments for the comparison of fertilizers, each experiment consisting of five blocks, and one plot per treatment per block. The treatments considered were:—

Experiment I.—No manure; 300 lb. superphosphate; 500 lb. superphosphate; 700 lb. superphosphate; 500 lb. superphosphate + 150 lb. nitrogen; 500 lb. superphosphate + 150 lb. potash; 500 lb. superphosphate + 150 lb. nitrogen + 150 lb. potash.

Experiment II.—300 lb. superphosphate; 300 lb. superphosphate + 300 lb. potash; 300 lb. superphosphate + 300 lb. nitrogen; 300 lb. superphosphate + 300 lb. nitrogen + 300 lb. potash; 300 lb. superphosphate + 150 lb. nitrogen + 150 lb. potash; 300 lb. superphosphate + 300 lb. nitrogen + 450 lb. potash; 500 lb. superphosphate + 300 lb. nitrogen + 300 lb. potash.

Experiment III.—No manure; 675 lb. Cresco Market Garden manure; 500 lb. superphosphate + 150 lb. nitrogen + 150 lb. potash; 650 lb. Commonwealth complete fertilizer.

The results were tested statistically by means of analyses of variance, the observations having first been transformed by the $\sqrt{x + \frac{1}{2}}$ transformation suggested by Bartlett (1), to allow for the non-normality of the data, and to make the variability in any class independent of the average value for that class. The tests showed that the fertilizers had produced no significant effects on the incidence of yellow dwarf, and that the diseased plants were distributed at random over the area, without any significant clustering in blocks.

Counts were also made in an experiment for comparing the effects of spreading superphosphate broadcast and by furrow. This trial was carried out in duplicate only, the layout being on the *a b b a* design, five of the 50-plant plots going to make up each replicate for each treatment. There was no difference between the numbers of infected plants under the two treatments.

In 1937-38, observations were made on fertilizer trials at Gunbower, Nathalia, Myrtleford, Markwood, and at two properties at Pomonal. The trials included seven randomized block experiments for comparing various combinations of superphosphate at levels of 200, 300, 500, and 700 lb. per acre, with nitrogen at 100, 150, and 300 lb., and potash at 100, 150, 200, and 300 lb., and castor meal at 500 lb. In most of these trials Hickory Pryor was used, though Dungowan Selection occurred in some at Pomonal. There were significant differences in the percentages of diseased plants in one experiment at Myrtleford and one experiment at Pomonal. The other five trials did not give significant results. Examination of the means for the significant group at Myrtleford showed that the combinations 500 lb. superphosphate + 100 lb. nitrogen, and 500 lb. superphosphate + 100 lb. potash, both had plots with a much higher percentage infection than the no manure, 300 lb. superphosphate, 500 lb. superphosphate, 700 lb. superphosphate, or 500 lb. superphosphate + 100 lb. nitrogen + 100 lb. potash. At Pomonal, the plots that received 300 lb. superphosphate + 150 lb. nitrogen had a much higher percentage infection than plots treated with no manure, 200 lb. superphosphate, 300 lb. superphosphate, 400 lb. superphosphate or 300 lb. superphosphate + 150 lb. nitrogen + 150 lb. potash. Although these results, to some extent, support one another, they were not borne out at the other centres, therefore no general relationship could be inferred from this group of experiments between any of the fertilizer combinations and the degree of infection. This group of analyses, when considered all together, suggests that the fertilizers had little, if any, real effect on the incidence of the disease.

In 1938-39, trial plots at Nathalia were used for yellow dwarf counts. For statistical purposes, these trials were divided into three separate experiments. The first was a simple randomized block

experiment involving two blocks, and the three treatments superphosphate alone, superphosphate with zinc sulphate, and superphosphate with magnesium sulphate. A plot consisted of a single row of 50 plants, the final stand being one or two short of 50. The analysis of variance test were made on the percentages of infection, and it was found that there were no significant differences.

The second trial was a combined fertilizer and seedbed soil treatment with the variety Orinoco Pride and was discussed earlier under (*c*), seedbeds and seedlings.

The third part of the fertilizer trials was a randomized block experiment, with five blocks, and seven fertilizers consisting of different combinations of superphosphate, blood manure, castor meal, and sulphate of potash. All the seed for this trial was treated with formalin. The analysis of variance showed that the variations in percentage infection between rows treated with the different fertilizer combinations just reached the low level of significance (i.e., probability of .05 that they could be exceeded by chance). Examination of the means of the percentages did not give any indication of association of high or low infection with any particular fertilizer, and as the level of significance was low, it seems likely that the differences obtained were fortuitous, and did not represent a genuine fertilizer effect. In this experiment, the block term also reached the 5 per cent. level of significance, and examination of the block means suggested some slight degree of patchiness in the infection.

At Myrtleford, in 1938-39, the variety Hickory Pryor was used in two randomized block experiments, planted on 17th October, for comparison of fertilizers. In the first experiment the treatments were the same as for Experiment I. of 1936-37. The second experiment was set out in a similar manner, but there were only four randomized blocks, each with seven treatments, for comparison of superphosphate at 300 and 500 lb. per acre, with 100 lb. nitrogen, and 100, 150, 300, and 450 lb. of potash. When observations were made on 2nd February, the height of the plants, including inflorescence, varied from 1 to 2½ feet. More than 99 per cent. of the plants were diseased. The fertilizers did not produce any significant effect on occurrence of yellow dwarf.

From these trials, conducted over a period of three years, and from observations made elsewhere and in other years, it can be concluded generally that fertilizer treatment had little, if any, effect on the incidence or the growth of diseased plants. As in the variety experiments, the most conspicuous differences in percentage infection were between different places in any one season, and between different seasons.

(*f*) *Time of planting.*

Differences in percentage infection by yellow dwarf due to time of planting have been observed, but such differences were of a seasonal nature. The transplanting period extends from October to December inclusive, and occasionally into early January, the early plantings being harvested in the summer months and the late plantings often being adversely affected by cool, autumn weather. Most of the transplanting is done during November, the first diseased plants usually appear towards the end of the month, becoming very obvious and numerous in

December. In some seasons, early and late-planted crops are less affected than others, probably because the early-planted crops have grown sufficiently to resist the worst effects of the disease, and because the late crops escape infection. The main infective period does not occur at the same time each year; it appears that both time and duration depend on seasonal conditions. In 1939-40 it was in December, in 1940-41 and in several previous years, it was in November. After the main infective period is past, comparatively few plants become diseased. Time of planting cannot, therefore, be relied on as a means of avoiding the disease, but the evidence so far available suggests that late-planted crops are usually less affected than early-planted crops. It might, therefore, be practicable to develop an early maturing variety that could be planted late enough to avoid the worst infection period and be ready for harvesting under favourable weather conditions.

4. Acknowledgments.

The authors wish to express their thanks to the tobacco officers of the Departments of Agriculture in New South Wales, Victoria, Queensland, and South Australia for their help when observations were being made on State experiment plots; and to Messrs. J. M. Allan, D. O. Norris, and G. H. Marks who assisted in the field work associated with collection of data.

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The Preparation of Emulsions for Coating Fruit and Vegetables.

*By S. A. Trout, M.Sc., Ph.D.**

Summary.

Thin films of suitable materials applied to the skins of many kinds of fruit and vegetables increase their storage life and decrease their rate of wilting. A convenient method of applying such coatings is by immersion of the product in aqueous emulsions of certain oils and waxes, but, in order to leave a clear, bright film, these ingredients must be as finely dispersed as possible.

Methods are described for preparing various oil emulsions and "colloidal" wax emulsions in which the hydrogen-ion concentration is adjusted for each particular wax by using alkaline solutions containing mainly sodium bicarbonate.

1. Introduction.

Many thin films of wax, oil, shellac, and other protective materials retard moisture loss from plant tissues, and this treatment is extensively used in the fruit industry. Wax films have been generally used in preference to the other protective materials as they give the fruit a brighter and more natural appearance. The fruits are coated by transferring wax from a slab by means of brushes, by spraying with molten wax or a solution of wax, or by immersion in an emulsion of wax in water.

For a number of years the Division of Food Preservation in co-operation with the New South Wales Department of Agriculture has been testing the effect of various protective wax coatings on moisture loss from citrus fruits, and wax emulsions have been mainly used because the method of application is simple and requires no elaborate machinery.

According to Platenius (1) most of the emulsions in commercial use in the United States are colloidal suspensions of paraffin and carnauba wax in water emulsified by oleic acid soaps of ammonia, sodium, potassium, or triethanolamine. The emulsions sometimes contain shellac, gum, resin, or mineral oil in addition to the dispersed wax. The commercial preparations used in Australia generally contain 20 to 24 per cent. total solids in the stock solution, including 3·5 to 7·5 per cent. soap. Commercial emulsions sometimes contain borax and vary considerably in their alkalinity. The neutral and weakly alkaline preparations containing 5 per cent. wax have considerably reduced weight loss in citrus fruits, but the more alkaline preparations have had no significant effect.

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* An officer of the Division of Food Preservation and Transport.

Until recently interest had been centred chiefly on the value of protective films for retarding moisture loss, but the possibility of using these coatings to prolong the storage life of fruit is now receiving wide attention.

The use of coating materials in solution is severely limited on account of the toxic effects on the fruit of the majority of solvents for waxes and most oils; it is necessary, therefore, to apply the latter materials as water-base emulsions.

In studying the effects on the storage behaviour of the plant materials, it is essential to know the exact composition of the emulsion used, because the effectiveness of the film is determined by the composition of both the dispersed wax or oil phase and the continuous aqueous phase.

2. The Preparation of Oil Emulsions.

There is no difficulty in preparing oil emulsions with oleic acid soaps of ammonia, sodium, potassium, triethanolamine, morpholine, turkey red oil, and many other emulsifying agents.

An emulsion containing 20 per cent. of oil may be prepared with approximately 1 per cent. soap. Smaller dispersed particles are obtained by allowing the soap to form during the emulsification process by adding the alkali to the water and the fatty acid to the oil phase. Larger particles are also broken down by homogenizing the emulsion.

Numerous tests have indicated that oil emulsions are more efficient in prolonging storage life than wax emulsions of similar concentration. One objection to their use commercially is the dull or unnaturally oily appearance of the fruit, but this can be improved to some extent by adding one part of a 20 per cent. solution of wax-free shellac in alkaline solution to five parts of emulsion. Emulsions of drying oils markedly taint the fruit and are therefore not recommended.

3. The Preparation of Wax Emulsions by Adjusting the Hydrogen-Ion Concentration of the Aqueous Phase.

The numerous emulsifying agents that have been used to prepare suspensions of wax in water are discussed in detail by Clayton (2). The methods described are in general use for the preparation of such products as cosmetics and polishes, but are not generally suitable for the preparation of emulsions for coating fruits.

The appearance of a wax film is affected by the size of the dispersed particles, and if the average size of the particles is greater than two or three microns in diameter the emulsion is opaque, and the dry film is dull and powdery. A clear and bright film is obtained when the average size of the dispersed particles is less than one micron in diameter and such preparations are termed "colloidal" emulsions. The only reference to their preparation is by Clayton, who states that "of scientific interest are specifications relating to the preparation of transparent emulsions by adjustment of the refractive indices of the liquid phases."

Such wax emulsions cannot be prepared simply by adding the molten waxes to "neutral" soap solutions and then homogenizing.

Donnan (3) showed that the interfacial tension against water of oils and hydrocarbons containing very small amounts of fatty acids is very greatly diminished by the presence of alkali in the water, and Hartridge and Peters (4) have shown that this fall in interfacial tensions is a function of the hydrogen-ion concentration of the water, commencing at pH 4·5 (approx.) and continuing up to about pH 10 when the interfacial tension becomes extremely small. The lower the interfacial tension at the time of emulsification the smaller will be the particle size of the dispersed phase. "Colloidal" emulsions of wax will be formed only if the solution of the alkali at the time of soap formation and emulsification is of a certain hydrogen-ion concentration, the critical value of which depends on the composition of the waxes being emulsified.

"Colloidal" emulsions of paraffin wax can be prepared with triethanolamine which has a pH value between 10 and 11 in water. The use of sodium carbonate gives "colloidal" emulsions with beeswax and lac wax, and a solution of borax and triethanolamine or sodium carbonate and sodium bicarbonate of pH 9·0 gives "colloidal" emulsions with carnauba wax and with mixtures of paraffin and carnauba waxes.

A solution of sodium bicarbonate and sodium carbonate of pH 8·8 is used to prepare "colloidal" emulsions of mixtures of paraffin and lac waxes.

Of special interest is the use of sodium chloride with sodium bicarbonate of pH 8·2 to prepare a "colloidal" emulsion of paraffin and beeswax mixtures. Similar results are not obtained by using only a sodium bicarbonate solution of the same hydrogen-ion concentration. Dubrisay (5) also found that sodium chloride increased the action of alkali in lowering interfacial tension.

An essential feature of the method of preparation is the formation of the soap simultaneously with the first stage of emulsification, and this is achieved by adding the alkali dissolved in a minimum quantity of water to a mixture of the fatty acid and molten wax.

It is believed that the control of hydrogen-ion concentration in these preparations by the use of sodium carbonate and bicarbonate, together with the use of sodium chloride has not previously been described. "Colloidal" emulsions of mixtures of paraffin wax with beeswax, carnauba, and lac waxes have been prepared by the above method, and it could probably be used for other waxes containing hydrophilic groups.

(a) The Preparation of Paraffin Wax Emulsions.

Since paraffin wax contains no active polar groups in the molecule, its emulsification is much more difficult than that of waxes such as carnauba, beeswax, and lac waxes which contain hydrophilic groups. Satisfactory emulsions of paraffin wax can be prepared with oleic acid and triethanolamine or morpholine using the following formula and method of preparation:—

Paraffin wax	112	parts by weight.
Oleic acid	38	" " "
Triethanolamine	18	" " "
Water	504	" " "

The triethanolamine is dissolved in 100 parts of hot water and then is slowly added with constant stirring to the molten mixture of paraffin wax and oleic acid. A smooth white cream is formed and the remaining hot water is added slowly, and the emulsion boiled for one minute.

The emulsion is a very stable, slightly viscous, opalescent liquid containing 25 per cent. of solids and 16·5 per cent. of wax by weight. It can be diluted with cold water and spreads easily on oranges and the more waxy surface of apples. The dry film is clear and fairly bright, and it increases storage life and retards weight loss effectively.

The emulsion contains one part of soap to two parts of wax, but one disadvantage in using such high concentrations of soap is the rather hygroscopic nature of the film. Emulsions containing only paraffin wax are manufactured in America, and in these preparations 40 to 50 per cent. of the total solids is soap. Such emulsions could be used if no other waxes were available, but very much less viscous emulsions giving clearer and brighter films can be prepared with smaller amounts of soap by mixing paraffin wax with a wax containing hydrophilic groups.

(b) The Preparation of Emulsions Containing Paraffin Wax Mixed with Waxes Containing Hydrophilic Groups

The number of waxes with hydrophilic groups from which emulsions can be manufactured commercially is limited by the availability of supplies, and it appears that only lac wax now being imported from India, and beeswax a product of Australia, can be used for this purpose.

The preparation of various commercial emulsions of paraffin wax and of paraffin and carnauba wax mixtures with other emulsifying agents, however, is protected by patent rights, but as far as is known "colloidal" emulsions of paraffin and beeswax and of paraffin and lac wax mixtures giving a bright and attractive film, increased storage life, and reduced moisture loss with fruits and vegetables have not previously been prepared.

The following formulae and method of preparation are, therefore, recommended for certain wax mixtures.

(c) The Preparation of Emulsions of Paraffin Wax and Beeswax.

"Colloidal" emulsions of paraffin wax mixed with 2, 3, 4, or 5 parts of beeswax can be prepared with oleic acid and a solution of sodium bicarbonate and sodium chloride.

Formulae.

Mixture.	Total Solids.	Wax.	Parts by Weight of—					
			Paraffin Wax.	Beeswax.	Oleic Acid.	Sodium Bicarbonate.	Sodium Chloride.	Water.
	%	%						
1 ..	24·5	20·0	92	46	22	6·6	2·2	521·2
2 ..	23·7	20·0	126	42	22	6·6	2·2	641·2
3 ..	28·7	25·0	168	42	22	6·6	2·2	599·2
4 ..	27·9	25·0	210	42	22	6·6	2·2	725·2

The paraffin wax, beeswax, and oleic acid are melted in a metal container and then a solution of sodium bicarbonate and sodium chloride in 100 parts of boiling water is added. The mixture effervesces through the liberation of carbon dioxide, and gentle heat and rapid and continuous stirring must be applied until the mixture begins to thicken and all the carbon dioxide is driven off. The mixture is then stirred with occasional heating until a clear jelly-like mass is formed which adheres fairly firmly to a glass stirring rod and forms a clear jelly-like film on it. About 25 per cent. of the remaining water is then added hot, and a greyish cream is formed. This mixture is stirred until it is homogenous and then the remaining hot water is added and the mixture stirred vigorously. The hot "colloidal" emulsion is thin, transparent, very opalescent and greyish in colour, but becomes less "colloidal", more viscous, and whiter in colour on cooling.

Accuracy in weighing the ingredients is essential, since insufficient alkali results in incomplete emulsification, whilst excess alkali precipitates the colloid.

(d) Properties of the Various Mixtures.

In commercial practice it is an advantage to have high concentrations of wax in the stock emulsion to reduce freight costs, and as mixtures 1 and 2 can only be prepared in 20 per cent. concentrations the other mixtures may be favoured by the trade. As the proportion of beeswax to paraffin wax is decreased, the emulsions become less "colloidal" and consequently less stable. These emulsions do not completely cover fruit with waxy skins and therefore the addition of a suitable spreading agent is frequently advantageous, and, moreover, may improve the stability of the emulsion.

(e) The Preparation of Emulsions Containing Mixtures of Paraffin Wax and Lac Wax.

Shellac contains approximately 4 to 5 per cent. of lac wax insoluble in alchohol but soluble in benzene. This wax is hard and brittle, and has a higher melting point and a lower acid value than beeswax.

The following formula is recommended for preparing an emulsion of 4 parts of paraffin and 1 part of lac wax:—

Paraffin wax	108	parts by weight.
Lac wax	27	" " "
Oleic acid	22	" " "
Sodium bicarbonate	4·5	" " "
Sodium carbonate (anhydrous)	0·8	" " "
Water	377·7	" " "
Percentage total solids	30·0	
Percentage wax	25·0	

The method of preparation is essentially the same as for a mixture of paraffin wax and beeswax. Although sodium carbonate is given as the anhydrous salt, there is no objection to using washing soda provided allowance is made for the water of crystallization.

The hot "colloidal" emulsion is very similar to the beeswax preparation, but becomes ivory-coloured on cooling and is more stable than a paraffin emulsion containing 25 per cent. of beeswax.

(*f*) *The Preparation of an Emulsion of Mixtures of Paraffin and Carnauba Waxes.*

An emulsion of paraffin and carnauba waxes can be prepared with oleic acid and a solution of sodium bicarbonate and sodium carbonate. The following formula is recommended:—

Paraffin wax	112	parts by weight.
Carnauba wax	28	„ „ „
Oleic acid	22	„ „ „
Sodium bicarbonate	4·5	„ „ „
Sodium carbonate (calculated as anhydrous)	1·5	„ „ „
Water	392	„ „ „
Percentage total solids	30·0	
Percentage wax	25·0	

The method of preparation is essentially similar to that used for beeswax and lac wax. The cold emulsion is greyish in colour, opalescent and very stable.

(*g*) *General Properties of the Emulsions.*

The amount of alkali used in the preparation of emulsions containing beeswax and carnauba should exactly neutralize the oleic acid, but less than the theoretical amount of alkali is used in preparing lac wax emulsions. The pH value of carnauba and beeswax emulsions is 8·6 compared with 8·2 for lac wax, and this slight alkalinity is probably due to hydrolysis of the soap.

In mixture 3 of the beeswax preparations 11·6 per cent. of the total solids is soap, whilst in the lac wax and carnauba wax emulsions 16·7 per cent. of the total solids is soap, compared with 20 to 38 per cent. in most commercial preparations.

4. The Spreading of the Emulsions.

These emulsions will completely cover the surface of potatoes, carrots, and any non-waxy surface, but spreading agents have to be added when they are used for fruits with waxy skins. It is an interesting fact that if additional soap is added during the preparation of the emulsions their spreading power is not improved, and the emulsions become more viscous.

Spreading agents should be added to the cold emulsions, and it is advisable to use non-alkaline spreaders to avoid excess alkali by hydrolysis on dilution which increases the rate of wilting of fruits and vegetables. Alkyl-naphthalene sulphonates are suitable spreading agents.

The one objection to the use of wax emulsions of low alkalinity is their tendency to break under commercial conditions, but whether this is caused by continuous agitation or by the leaching out of small amounts of acid from the fruit is not clear. Good emulsions can be broken by agitation alone, as the emulsifying agent or protective colloid leaves the wax/water interface by preferential absorption at the air/water interface. Emulsions can also be broken by the addition of

acids or electrolytes which "salt out" the soap and destroy the interfacial film, but certain protective colloids are readily absorbed by "colloidal" suspensions and protect them from precipitation by electrolytes. If a 0·1 per cent. solution of an alkyl-naphthalene sulphonate or other sulphated or sulphonated spreading agent is added to emulsions, they are protected from precipitation by acids and electrolytes and can only be broken by alcohol.

5. The Incorporation of Fungicides in Emulsions.

Borax is incorporated in some of the commercial emulsions for controlling mould wastage due to *Penicillium*, which is the main rot-producing organism in citrus fruits, but excessive alkali tends to counteract the beneficial effect of the emulsion in retarding water loss. Experiments on the effects of various non-alkaline fungicides added to the emulsions are in progress.

6. Discussion.

Although coatings are in many cases very effective in prolonging storage life, they cannot be applied indiscriminately to all fruits as, in some cases, severe internal disorders and fermented flavours are produced. Each variety has to be tested before a particular coating can be recommended. The experiments have been carried out on a laboratory scale by dipping small quantities of fruit and drying it on trays.

There are, however, certain technical problems associated with waxing fruit commercially, and to aid in their solution a commercial waxing plant is now being erected at Homebush.

7. Acknowledgments.

The author wishes to record the valuable help in carrying out these studies given by Mr. E. G. Hall, B.Sc.Agr., of the New South Wales Department of Agriculture, and Mr. E. Fisher, the author's assistant.

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Scientific Papers from the Division of Food Preservation and Transport Published Elsewhere than in the Council's Publications.

In a previous issue (11: 278, 1938) an article appeared discussing scientific papers for which officers of the Council's Division of Food Preservation and Transport were responsible, but which had been published elsewhere than in the Council's publications. The article that follows brings the former list of such publications up to date.—Ed.

A. General.

Vickery, J. R. (1940).—The Work of the Division of Food Preservation and Transport of the C.S.I.R. *Aust. J. Sci.*, **2**: 156.

An outline of the history and research programme of the Division.

B. Meat.

Riddle, A. R. (1940).—C.S.I.R. and Chilled Beef. *Qld. Country Life*. 1st August.

Slaughter floor hygiene and gas storage, which have been investigated by the Council, have made it possible to prevent microbial spoilage of chilled beef long enough to ship it to England. Present studies are concerned with the bloom of the chilled beef which is influenced by the quality of the animals slaughtered and the conditions of storage. The quality of the beef may depend on breed, early or late maturity, nutrition, and resting before slaughter. The storage conditions requiring investigation are air circulation and relative humidity during ship-board storage, bruising and chafing which may result from movements of the ship, and the type of wraps used on the beef.

Riddle, A. R. (1941).—C.S.I.R. Reviews Current Beef Problems. *Qld. Country Life*, 31st July.

Discusses the present transport difficulties, both overseas and in Australia, and describes methods of boning and packaging meat to conserve cargo space.

In Co-operation with The Low Temperature Research Station, Cambridge.

Scott, W. J., and Haines, R. B. (1940).—Anaerobic Organism Associated with "Bone-taint" in Beef. *J. Hygiene*, **40**: 154.

In the past, "bone-taint" in beef has been a widespread source of spoilage. It has, however, been virtually eliminated where the dressed sides can be rapidly cooled in refrigerated hanging rooms. In the summer of 1938, an outbreak occurred in Norfolk, where cattle were killed in a local slaughter-house and the sides hung to cool and set for about 24 hours above the killing floor before they were transported to a refrigerator. Some two or three days later, on cutting up the hind quarters, a most offensive odour emanated from the deep parts of the tissues near the bone, although the outer part of the quarters appeared normal.

By laboratory examination a large spore-producing rod was found to be present in great numbers. It was strictly anaerobic and grew poorly on most culture media. It was identified as a *Clostridium*, resembling in some ways *Cl. oedematiens.*

Later the hip joints of apparently normal quarters of beef were examined for their bacterial contents in an attempt to throw light on the mode of infection in "bone-taint". They were found to be sterile usually two to three days after slaughter.

C. Fish.

In Co-operation with the Fisheries Research Board of Canada.

Collins, V. K., Kuchel, C. C., and Beatty, S. A. (1941).—Studies of Apples. Effect of Picking Maturity, Delayed Storage, and Wrappers. *J. Dept. Agric. Vict.*, **37**: 77.

In a search for practical objective standards for the freshness of fish, its buffering capacity has been found useful. In this paper it is shown that cod muscle press-juice during storage shows a decrease, and later an increase, in buffering capacity between pH 6·0 and pH 3·4, both due to bacterial action. Reduction of trimethylamine oxide causes the decrease, and oxidation of lactic acid to acetic acid slightly lessens the effect. The increase is possibly entirely due to hydrolysis of protein by bacteria, and, as it occurs after the fish is definitely spoiled, it is useless for the detection of early deterioration. The decrease in buffering capacity occurs during the onset of spoilage so it is useful in estimating the state of preservation, but it is not as reliable as the determination of dimethylamine or of trimethylamine.

D. Fruit.

In Co-operation with the Department of Agriculture of Victoria.

Tindale, G. B., Huelin, F. E., and Trout, S. A. (1938).—Victorian Plums and Peaches—Cool Storage and Export. *J. Dept. Agric. Vict.*, **36**: 609.

Stages of maturity for picking plums and peaches, storage life at different temperatures, fungal rotting in stored peaches, and respiration during cool storage are described.

Neither for local storage nor for export is the picking of immature plums and peaches recommended. Such fruit is quite green and hard and very sour. In mature plums and peaches, which develop maximum juice and flavour on ripening, the flesh and ground colour have changed to a whitish green, and very slight softening has occurred. Mature peaches can also be distinguished by the high development of blush, but plums cannot be distinguished in this way, as some blue and red varieties are fully blushed when only partly grown. The juice of mature plums and peaches is higher in soluble solids and lower in total acid content than that of immature fruits. There was no significant difference with maturity in the iodine titration or the catalase content of the juice.

The storage life at 32°F. is limited by disorders which develop after removal to ripening temperatures, while at the minimum ripening temperature, it is limited by overripeness. It has been found that mature plums can be kept for a considerable period by storing initially

at 32°F. and then transferring to the minimum ripening temperature, which varies from 40° to 46°F., according to the variety. Similar results have been obtained with mature peaches.

The control of fungal rotting of peaches in storage (particularly that due to brown rot) is primarily attained by reducing infection in the orchard.

At 45° to 55°F. peaches are fully ripe when they have reached the climacteric of respiration, but plums do not become fully ripe till much later. The storage life at 32°F. is not more than half the time necessary to reach the climacteric.

Tindale, G. B., and Huelin, F. E. (1939).—Superficial Scald in Apples. Effect of Picking Maturity, Delayed Storage, and Wrappers. *J. Dept. Agric. Vict.*, **37**: 77.

Granny Smith, Stewart, and Delicious apples are cool-stored in considerable quantity throughout Victoria, and severe losses have been experienced by growers owing to the development of superficial scald either during or shortly after removal from storage. This disorder generally occurs as a light-brown discolouration of the skin, affecting green apples in particular. The area affected is usually mottled rather than wholly brown, and in this respect differs from soft scald of the Jonathan. The flesh beneath the scalded area is not affected until the scald becomes very bad. Plain wraps and delayed storage were found to be of little use in controlling superficial scald, but oil wraps gave complete control.

Huelin, F. E., and Tindale, G. B. (1940).—Cool Storage of Plums. Progress Report. *J. Dept. Agric. Vict.*, **38**: 247.

Several varieties of plums were picked at two stages of maturity, and were stored at 32°F. in air and in "gas" (5 per cent. and 10 per cent. carbon dioxide). They were subsequently ripened at both 45°F. and 60°F. and the storage life at 32°F. determined. This is the maximum storage period at 32°F. if normal ripening is to occur when the plums are transferred to the ripening temperature. For most varieties gas storage definitely decreased the storage life, and with some varieties even as little as 2 per cent. of carbon dioxide proved harmful.

Huelin, F. E., and Tindale, G. B. (1941).—Gas Storage of Peaches. *J. Dept. Agric. Vict.*, **39**: 34.

Influence of maturity on gas storage was studied, the effect of different storage atmospheres and temperatures, and the best temperatures for subsequent ripening of the peaches.

The storage life of peaches in air at 32°F. is little affected by picking maturity, but shows a considerable seasonal variation. On the other hand, the effect of gas storage is closely related to maturity. Peaches picked in a firm condition generally have their life at 32°F. increased by gas storage, but the life of softer peaches is unaffected or decreased. Where the life is increased by gas storage, the maximum effect is obtained in atmospheres containing 8 to 10 per cent. of carbon dioxide and 13 to 11 per cent. of oxygen (by reduced ventilation). Increases in the storage life as a result of gas storage have been obtained only when the peaches have been subsequently ripened at 60° to 65°F. Any advantage of gas storage is lost if the peaches are subsequently ripened

at 45°F., and for this reason is not recommended for use in exporting peaches to England as this would be the prevailing temperature when the peaches were landed.

TINDALE, G. B., and HUELIN, F. E. (1941).—Refrigerated Gas Storage of Fruit. *Fruit World Annual*, Jan. 1941, p. 47.

This article describes the accidental discovery of gas storage at the Cambridge Low Temperature Research Station while the effect of carbon dioxide on brown heart in apples was being studied. Although brown heart develops when the carbon dioxide from the respiration of the fruit accumulates until it forms 10 per cent. of the atmosphere, 5 to 7 per cent. was found to have the desirable effect of increasing the storage life in certain varieties.

Work has been in progress for five years at the Government Cool Stores, Melbourne, to discover which of the different varieties of fruits grown here would benefit by gas storage. Jonathan apples, also Granny Smith, Stewarts, and Democrat, picked at an early stage of maturity, have been found to benefit, also all varieties of pears, but for other fruits gas storage is not beneficial, or has actually brought about disorders.

In Co-operation with the Botany School, University of Sydney, and the Department of Agriculture of New South Wales.

TROUT, S. A., ROBERTSON, R. N., HALL, E. G., and HACKNEY, FRANCES (1941).—Effect of Skin Coatings on Apples. *Nature*, **147**: 27.

Apples were coated by dipping in solutions or emulsions containing various proportions of oil, wax, and shellac. The relationship of treatments, internal oxygen and carbon dioxide, and external respiration was studied over a range of temperatures.

Lesions identical with "brown heart" developed in Granny Smith apples in which the internal oxygen concentrations were reduced to approximately 3 per cent. by a skin coating but in which the internal carbon dioxide concentration was not significantly increased. This is interesting because previously brown heart has generally been associated with fruit stored in atmospheres containing considerably more carbon dioxide than is present in air.

In Co-operation with the Low Temperature Research Station, Cambridge.

HUELIN, F. E., and BARKER, J. (1939).—Effect of Ethylene on the Respiration and Carbohydrate Metabolism of Potatoes. *New Phytologist*, **38**: 85.

The effect of ethylene on the respiration and sugar content of potatoes at 15°C. was determined after varying periods of storage at this temperature. The characteristic effect was an increase in the respiration to a maximum during the first two days after exposure to ethylene, followed by a fall in the respiration to an adjusted level above the control value. Increases in respiration were obtained in a concentration of one part per million of ethylene. The response increased with rising concentration up to one part per thousand. Etyhlene had only a small or no effect on the respiration of potatoes shortly after harvesting, but the effect increased in magnitude with continued storage.

Attempted Transmission of *Anaplasma marginale* Theiler by Biting-Flies.

By I. M. Mackerras, M.B., Ch.M., B.Sc., M. J. Mackerras, M.B., M.Sc.,† and C. R. Mulhearn, B.V.Sc.‡*

Summary.

Experiments have been carried out with two common biting flies in an attempt to confirm the findings of American workers that anaplasmosis can be transmitted mechanically by blood-sucking Diptera.

Tabanus circumdatus Walk. (the common March fly) failed to transmit *Anaplasma marginale* mechanically; and *Stomoxys calcitrans* Linn. failed to transmit it either mechanically or cyclically.

The disease was transmitted by puncture with a contaminated needle, as well as by subcutaneous and intravenous injections of infected blood.

The blood sometimes became infective as early as the second day of the incubation period.

1. Introduction.

Recent work on the blood parasites of cattle in Australia has been described by Legg (1933 to 1939), who has also reviewed earlier investigations in this country. In 1933, the late Dr. J. A. Gilruth invited us to co-operate with Dr. Legg in certain aspects of the work. He did so, because our laboratories were situated in an area far removed from any centre of tick infestation or enzootic anaplasmosis, and because we had insectaries in which cattle could be kept free from blood-sucking flies or ectoparasites. We were first asked to test the possibility of transmitting *Anaplasma marginale* by biting flies, and later to handle the introduction of *Anaplasma centrale*. The results of the former studies are presented in this paper.

The general association of anaplasmosis with tick infestation, and the numerous records of experimental transmission of the disease by means of ticks, leave little room for doubt that they are the normal vectors. To date the following thirteen species have been definitely incriminated in various parts of the world:—*Boophilus annulatus* Say., *B. decoloratus* Koch, *B. microplus* Canest., *Rhipicephalus simus* Koch, *R. bursa* C. & F., *R. sanguineus* Latr., *Dermacentor variabilis* Say., *D. andersoni* Stiles, *D. occidentalis* Neum., *D. albipictis* Packard, *Hyalomma lusitanicum* Koch, *Ixodes ricinus* Linn., *I. scapularis* Say. In Australia, Ferguson (1925) has recorded seven species of ticks as having been taken on cattle, viz.:—*Boophilus australis* Full., *Haemophysalis bispinosa* Neum., *H. leachi* Aud., *H. papuana* Thor., *H. longicornis* Neum., *Amblyomma triguttatum* Koch, *Aponomma trimaculatum* Lucas. Of these, *B. australis* and *H. bispinosa* are normal cattle parasites; the others are of rare, accidental occurrence and can be excluded from practical consideration as vectors of cattle diseases. In addition, *Rhipicephalus sanguineus* Latr., with which Rees (1930A)

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† An officer of the Division of Economic Entomology (on extended leave).

‡ An officer of the Queensland Department of Agriculture and Stock.

has experimentally transmitted anaplasmosis in America, occurs on dogs in Queensland and the Northern Territory, and has been recorded by Fielding (1926) from cattle. There seems to be little doubt that *B. australis* transmits anaplasmosis in Queensland, but it would be desirable to determine whether *H. bispinosa** can do so also, for this species occurs in eastern New South Wales in country far south of that at present occupied by *B. australis*.

That *A. marginale* can be transmitted by other agents besides ticks is now well known. The method of direct blood inoculation is universally used in experimental work, and Taylor (1935) has successfully transmitted *A. marginale* to healthy cattle with as little as 0·1 ml. of infected blood, although he failed with 0·01 ml., and Stiles (1936) produced the disease with 0·025 ml. given intradermally. Anaplasmosis has also been transmitted to clean cattle by a goad (Descazeaux, 1924), by dehorning instruments (Hilts, 1928; Stiles, 1931 and 1936), by a lancet (Rees, 1930B), and by hypodermic needles (Boynton, 1934), the instrument in each case being simply contaminated by use on infected animals; and by the injection of contaminated vaccine (Le Clainche, 1930).

The disease occurs in the United States of America well beyond the present limits of the cattle tick, *Boophilus annulatus*, and besides other species of ticks, various blood-sucking insects have been suggested as possible vectors, but the only positive results so far recorded are those of the American workers, Sanborn, Stiles, and Moe (1930, 1932, and 1933), who succeeded in transmitting *A. marginale* mechanically by several species of Tabanidae, and Sanders (1933), who succeeded with *Tabanus fumipennis* and *Stomoxys calcitrans*. Descazeaux (1924) considered that the spread of anaplasmosis in tick-free regions in Chile was due to biting flies.

On the other hand, Lignières (1914) considered it unlikely that anaplasmosis was transmitted by *Stomoxys calcitrans* in the Argentine. He had observed that healthy animals kept in the same stalls as sick ones and freely bitten by stable flies never acquired the disease. Parodi (1917) also concludes for similar reasons that *Stomoxys* and Tabanids do not transmit the disease there. Brumpt (1931) made similar observations on animals kept in stables of the Faculty of Medicine, Paris, where *Stomoxys calcitrans* is abundant; also he observed that no spontaneous cases occurred in Normandy, where clean and infected animals had been running together for some years. More recently, Taylor (1935) working in England, failed experimentally to transmit *A. marginale* mechanically by the bites of *Haematopota fluvialis* and *Stomoxys calcitrans*, and in Montana, U.S.A., Morris, Martin, and Oglesby (1936) failed with *Lyperosia irritans*, *Tabanus atratus*, and *T. fuscicostatus*, although Morris had obtained a positive result with *T. atratus* in a preliminary experiment, which, however, was not considered fully satisfactory.

In the course of his work at Townsville, Legg observed that cases of anaplasmosis sometimes developed under conditions that rendered transmission by ticks unlikely, so the question of insect transmission in Australia was taken up. This question is of importance, not

* *H. bispinosa* failed to transmit *Babesia bigemina* in a large series of experiments (Legg, 1926).

only as it may affect the conduct and results of experimental work in Queensland, but also in its bearing on the distribution and control of the disease. The number of possible vectors among the blood-sucking Diptera alone is far too large for all to be tested, so attention was concentrated on two species, which occur both at Townsville and Canberra, which were considered likely to be efficient mechanical transmitters, and which were comparatively easy to handle under experimental conditions. These were *Stomoxys calcitrans* Linn. and *Tabanus circumdatus* Walk.

2. Materials and Methods.

Our strain of *A. marginale* was obtained from Legg, who had established a pure infection in his Bovine No. 207, from which he sent us citrated blood. This blood was inoculated into our Nos. 201 and 202 on the 7th June, 1933, and both calves passed through typical mild attacks of anaplasmosis. These, and other calves inoculated from them, were used as donors in the various experiments. This strain of *A. marginale* has now been passed through 35 bovines at Canberra, and no other species of blood parasite has appeared in their blood as a result of the inoculation. Moreover, some of these animals have been infected subsequently with *Anaplasma centrale*, *Babesia bigemina*, and *Theileria mutans*, to all of which they proved to be susceptible. The purity of the strain may, therefore, be taken as proved.

The calves used were about six to nine months old, and were bred locally. They were kept on regular temperature and blood-film examination, and their clinical condition was carefully watched. In all experiments the donors were used when they showed moderate to large numbers of *A. marginale* in the peripheral blood. The infected donors and clean recipients were kept in separate insectaries under strict quarantine conditions. At first, the flies were carried from one insectary to the other, and the donor and recipient calves never came into contact, but later the calves were brought together in an insectary cubicle for two to three hours each day when the flies were being fed. A control calf was kept in contact with the donors.

Stomoxys calcitrans persists in Canberra in the winter, and it was used in the first series of experiments in July, 1933. A few of the flies used were captured, but most were bred from larvae and puparia collected in manure heaps. Both adults and early stages were kept in cages in a room maintained at a temperature ranging from 70°F. to 75°F. and a relative humidity of about 70 per cent., and illuminated solely by a 1,000-watt gas-filled electric light. Under these conditions, all stages did well, the flies lived up to 45 days, and a second generation was bred without difficulty from the flies that emerged in the cages. When not required for feeding on the calves, the flies were allowed access to guinea-pigs, which they attacked readily.

For the feeding experiments, the flies were transferred to 6 in. x 1¼ in. glass tubes. They were immediately taken to the insectary and returned to the warm room as soon as feeding was completed. Small areas of skin along each side of the back of donor and recipient were closely clipped with scissors, the plug was removed, and the open mouth of the tube applied to a clipped area, so that the flies could rest directly on the skin. A depilatory was used once, but the flies did not seem to

be able to obtain a satisfactory grip on the completely bald skin, and did not feed well. A dark sleeve placed over the upper part of the tube quickly induced the flies to drop on to the skin where they immediately commenced feeling about with the tip of the proboscis. Having selected a suitable spot, they proceeded to bore through the skin, frequently using the hairs to obtain a purchase with their legs. The calf reacted to the pricking by local shiverings and twitchings, and sometimes by kicking, which is not surprising, considering the quality of the *Stomoxys* bite on the human skin. Once through the skin, the flies settled down to a more leisurely feed, and were not easily disturbed, several sharp slaps on the skin close to the tube often being necessary in order to dislodge them before they had completely filled up. There was no difficulty in detecting whether each fly actually pierced the skin, nor in observing the first sign of swelling of the abdomen. When fully fed, the abdomen was greatly distended, and the contained blood could be seen clearly through the thin pleural membrane at the sides. Often the flies were so heavy that they could scarcely fly. The flies defaecated altered blood several times in the course of a feed, but were not observed to regurgitate fluid at any stage.

In the mechanical transmission experiments, the flies were used singly in tubes. Each fly was allowed to feed on the infected donor until the abdomen definitely commenced to swell; it was then transferred as quickly as possible to the recipient calf and was allowed to complete its feed. Sometimes a donor was pierced two or three times by a fly before the stage of abdominal swelling was reached, and sometimes a recipient was pierced more than once before the feed was completed, but usually the donor and recipient were each only pierced once. The time interval, from the moment the proboscis was withdrawn from the donor to the moment it actually entered the recipient, was read by means of a conveniently-placed clock with a large second hand, and was recorded on the tube. In the cyclical transmission experiments, up to five flies were used in a tube, and each was allowed to complete its feed.

The life history of *Tabanus circumdatus* is still unknown, so the Tabanids used were captured flies, collected in the mountains near Canberra, where they are abundant in the autumn. Many were collected on ourselves, but a quiet horse was a very useful additional bait for obtaining large numbers of flies. They were brought to the laboratory in cages and were used the same day, as it was found that even in 24 hours there was a high mortality and few of the surviving flies would bite.

Thermohygrograph records were kept in the insectaries throughout the experiments.

3. Experiments with *Stomoxys calcitrans* Linn.

(a) Attempts at Mechanical Transmission.

Experiment 1.—Calf No. 206 received 99 bites, distributed over a period of five days (1st-5th July, 1933). The intervals between feeding on No. 201 (donor) and on No. 206 (recipient) were from ten minutes to five hours, but the great majority of the bites occurred at considerably less than five hours' interval. The temperature in the insectary

was low during the periods when flies were being fed, ranging from 48°F. to 59°F. The relative humidity was usually high (50-90 per cent.).

No. 206 remained in good health, and no abnormal fluctuations of temperature occurred. Blood examinations were negative for 109 days from the first and 105 days from the last bite, when cross-inoculation was performed. Blood from No. 206 was withdrawn (on 18th October, 1933) and 15 ml. were injected intravenously into a clean calf, No. 216, and 10 ml. from No. 208, showing numerous *A. marginale*, were injected intravenously into No. 206. A mild infection followed, after an incubation period of seven days. No. 216 died from inanition due to intractable diarrhoea on the nineteenth day after inoculation. No parasites had appeared in its blood, and on the day before its death 20 ml. of blood were withdrawn and injected intravenously into No. 218. The blood of this animal was examined for 84 days after inoculation, but no parasites were detected.

Experiment 2.—Calf No. 207 received 417 bites, distributed over a period of eight days (7th-14th July, 1933). The intervals between feeding on the infected calf No. 202 and feeding on No. 207 varied from 5 minutes to 5 hours 51 minutes. More than half the bites occurred at less than two hours' interval. External temperature (46°-66°F.) and relative humidity records in the insectary were similar to those in Experiment 1. On the 62nd day from the last bite the calf was splenectomized. Blood was examined daily for 74 days from the last bite, i.e., until twelve days after splenectomy, when the calf died from post-operative pneumonia, but no parasites were detected.

Experiment 3.—Calf No. 211 received 252 bites, distributed over a period of ten days (9th-18th August, 1933). The intervals between feeding on the infected calf No. 208 and on No. 211 were from 17 seconds to 9 minutes 40 seconds, the intervals for 148 bites being less than one minute. Temperatures in the insectary were low, 47°-62°F., and the relative humidity high (50-90 per cent.). The temperature chart and blood films of the calf remained normal, and on the 27th day from the last bite splenectomy was performed, but parasites did not appear in the blood. Cross-inoculation was performed 53 days after splenectomy, 38 ml. of blood from No. 211 being withdrawn and injected intravenously into a clean calf, No. 218; 18 ml. of blood from No. 203, showing moderate numbers of anaplasms were then injected intravenously into No. 211. Incubation period in No. 211 was eight days, and a severe infection followed, causing the death of the calf on the nineteenth day after inoculation. The blood of No. 218 was examined for 83 days after inoculation, but no parasites appeared.

Experiment 4.—Calf No. 224 received 29 bites on 9th and 10th March, 1934, from flies which had fed on No. 203, showing moderate numbers of *A. marginale*. External temperatures were high (80°-98°F.) and relative humidity low (55-17 per cent.). On 3rd, 4th, and 5th April, 1934, calf No. 224 received 209 bites from flies that had fed on No. 215, showing numerous *A. marginale*. The interval in all these bites was less than one minute; flies that did not begin to feed again within this time being discarded. The minimum interval was 9 seconds. Temperatures in the insectaries were fairly high (65°-70°F.), relative humidity varied a good deal (75-25 per cent.). The blood

of No. 224 remained negative for 95 days from the last bite and 122 from the first, when cross-inoculation was performed, 20 ml. of blood being withdrawn from No. 224 and inoculated intravenously into a clean calf, No. 231; 20 ml. from No. 203 (a carrier) were injected intravenously into No. 224. The incubation period in No. 224 was 26 days, and a fairly severe attack of anaplasmosis followed. No. 231 remained free from parasites up to its death 63 days later.

(*b*) *Attempts at Cyclical Transmission.*

Experiment 5.—Calf No. 204 received 288 bites, distributed over 13 days. The intervals between feeding on the infected calf No. 202 and on No. 204 were from 2 to 9 days. Blood examinations remained negative for 103 days from the first and 90 days from the last bite, when cross-inoculation was performed (18th October, 1933), 15 ml. of blood were withdrawn from No. 204 and injected intravenously into No. 216. No. 204 then received intravenously 10 ml. of blood from No. 208, which showed large numbers of anaplasms. The incubation period in No. 204 was 7 days, and a moderately severe attack of anaplasmosis followed. The subsequent history of calf No. 216 is given under Experiment 1.

Experiment 6.—Calf No. 205 received 586 bites distributed over a period of 39 days. The intervals between feeding on the infected calves Nos. 201 and 202 and on No. 205 were from 8 to 42 days. The blood of No. 205 remained negative, and 80 days from the last bites 18 ml. of blood from it were injected into a clean calf, No. 218. This animal remained free from anaplasmosis until its death 83 days later. After 122 days had elapsed from the first and 84 days from the last bite, No. 205 received 14 ml. of blood from No. 203, showing rather scanty *A. marginale*. The incubation period in No. 205 was 8 days, and a moderately severe attack of anaplasmosis followed.

Experiment 7.—Calf No. 209 received 1,239 bites distributed over a period of 40 days. The intervals between feeding on the infected calf No. 202 and on No. 209 were from 8 days to 42 days. Blood examinations remained negative until the 70th day from the first bites, and the 31st from the last bites. The calf was then splenectomized, but died during the operation. Blood was withdrawn immediately and inoculated into No. 212 subcutaneously. The blood of No. 212 was examined regularly for 57 days, but no anaplasms appeared.

4. Experiment with *Tabanus circumdatus* Walk.

Experiment 1.—Calf No. 223 received 119 bites, the intervals between feeding on the infected calf No. 203 and on No. 223 varied from 10 seconds to 4 minutes 2 seconds; 103 bites occurred at an interval of less than one minute. No. 203 showed moderate numbers of anaplasms in the blood (1-4 per oil-immersion field). The temperature in the insectary remained high during the time the flies were being fed (81°-98°F.) and the relative humidity low (42-17 per cent.). No. 223 remained free from anaplasms, and, on the 91st day from the last and the 94th day from the first bite, cross-inoculation was performed. Blood was withdrawn from No. 223, and 40 ml. were injected intravenously into No. 231, and 20 ml. of blood from No. 205 were inoculated subcutaneously into No. 223. No. 205 had been through a

typical attack of anaplasmosis in November, 1933; *A. marginale* was last noted in the blood on 8th December, 1933, i.e., six months previously. The blood, however, was non-infective, as No. 223 did not develop the disease, although subsequently shown to be susceptible. The spleen of No. 223 was removed on the 40th day after inoculation from No. 205, and on the 131st day after the last bites. No relapse followed, and 4 weeks later (i.e., 158 days from the last bites) it was given 20 ml. of blood intravenously from No. 224 (showing numerous anaplasms). Incubation period in No. 223 was 8 days, and a particularly heavy infection followed. The calf died from anaplasmosis and pneumonia on the 19th day after inoculation with virulent blood.

These experiments are summarized in Table 1.

TABLE 1.

Expt.	Transmission.	Recipient.	No. of Bites.	Time Interval.*	External Temperature.	Period Allowed for Incubation.	Result.
					deg. F.	days.	
			(i) Experiments with *Stomoxys calcitrans*.				
1	Mechanical	206	99	10 mins.—5 hrs.	48–59	105–109	—
2	Mechanical	207	417	5 mins.—5 hrs. 51 mins.	46–66	74–81	—
3	Mechanical	211	252	17 secs.—9 mins. 40 secs.	47–62	80–89	—
4	Mechanical	224	238	9 secs.—60 secs. . .	65–98	95–122	—
5	Cyclical . .	204	288	2 days 9 days . .	46–66	90–103	—
6	Cyclical . .	205	586	8 days—42 days	45–65	84 122	—
7	Cyclical . .	209	1,239	8 days—42 days	45–65	31–70	—
			(ii) Experiment with *Tabanus circumdatus*.				
1	Mechanical	223	119	10 secs.—4 mins. 2 secs.	81–98	158–161	—

* The time interval refers to the time elapsing between withdrawal from donor and piercing recipient.

5. Experiments with Needles.

In the hope that some light might be thrown on the failure of the biting flies to transmit *A. marginale* mechanically, nine needle experiments have been carried out.

Experiment 1.—A clean calf, No. 225, was stabbed in the back 20 times with a diamond-shaped blood-smear needle, each stab alternating with a stab in the back of an infected calf, No. 215, which showed numerous *A. marginale* in the blood. The skin over an area of the back of both calves was shaved, the needle pierced through the skin each time, frequently drawing blood, and the interval between withdrawing the needle from the infected calf and plunging it into the clean calf was from 1-2 seconds for each stab. The recipient developed anaplasmosis after an incubation period of 31 days.

Experiment 2.—Another clean calf, No. 229, was stabbed in a similar manner 20 times, but the needle was held in the air for 10 seconds each time after withdrawal from the donor before it was plunged into the clean animal. The first 15 stabs were made with a diamond-shaped

blood-smear needle. The point of this needle then broke off, and remained embedded in the skin of No. 229. The last five stabs were made with a large triangular needle. No anaplasms appeared in the blood of No. 229, and 90 days after the stabs, cross-inoculation was performed, 20 ml. of blood being withdrawn for injection into a clean recipient and 20 ml. from No. 225 (a carrier) were given intravenously to No. 229. After an incubation period of 12 days, No. 229 developed a fairly severe infection.

Experiment 3.—This experiment was in all respects similar to the previous two, except that the needle used was guarded, so that it could penetrate only 2 mm. into the skin, this amount being the maximum possible penetration of *T. circumdatus* and longer than the possible penetration of *S. calcitrans*. A fine hypodermic needle was used held firmly in artery forceps. At 2 mm. only the flattened distal part, but none of the tubular part of the needle, enters the skin. Blood appeared at many of the punctures. The time interval between withdrawal from the donor and piercing No. 230 (the clean animal) was 1 to 1½ seconds. The number of stabs was 20. No anaplasms appeared in the blood of No. 230; on the 83rd day cross-inoculation was performed, using the same recipient as in Experiment 2. No. 230 developed moderately severe attack of anaplasmosis after an incubation period of 14 days. The recipient of the blood from Nos. 229 and 230 remained negative.

Experiments 4-6 were essentially a repetition of Experiments 1 to 3 respectively, but the needle used for the unguarded punctures was a large, stout, triangular one, and the operation was carried out in a crush in the open, whereas the previous three experiments had been done inside the insectary. All three animals remained free from blood parasites, and on the 51st day received infected blood intravenously. All developed anaplasmosis.

Experiments 7-9 were a repetition of Experiments 4 to 6 respectively. In these the animals were observed for 105 days and then cross-inoculation was performed. All three developed anaplasmosis; while the recipient of their pooled blood remained negative.

The results of these experiments are set out in Table 2.

TABLE 2.

Number of Animal.	Type of Needle Puncture.	Needle.		Subsequent Inoculation with *A. marginale* blood.	
		Result.	Incubation.	Result.	Incubation.
			days.		days.
225	Immediate, unguarded	+	31	..	..
262	Immediate, unguarded	—	51	+	17
452	Immediate, unguarded	—	105	+	17
230	Immediate, guarded	—	83	+	14
A222	Immediate, guarded	—	51	+	12
808	Immediate, guarded	—	105	+	17
229	Delayed, unguarded	—	90	+	12
D371	Delayed, unguarded	—	51	+	17
803	Delayed, unguarded	—	105	+	17

6. Discussion.

All but one of our tranmission experiments with *A. marginale* failed, and the question arises as to whether the failures were due to the agents used—biting flies, or needle under certain conditions—being unsuitable for the purpose, or whether they were due to any fault in the methods employed.

(*a*) *Were the experiments adequate?*

(i) With *Stomoxys calcitrans* we made four attempts at mechanical transmission and three attempts at cyclical transmission; with *Tabanus circumdatus* we made one attempt at mechanical transmission. These numbers are small, but we consider that, had these flies been capable of transmitting our strain of *A. marginale* under insectary conditions, they would have done so in two at least of the *Stomoxys* experiments and in the *Tabanus* experiment. In this connexion, it may be noted that the American workers, Sanborn and his co-workers, and Sanders, obtained uniformly positive results, under conditions which appear from their descriptions to have been not more favourable for transmission than were ours.

(ii) Our donors were all in approximately the same stage of infection, that between the time when the parasites have increased to two to four per oil-immersion field and the time when the blood reaction is well-established, a period of about seven days. That blood is infective at this stage has been thoroughly established by transmission experiments with ticks and flies in other parts of the world, and for our strain by the positive needle experiment and numerous transmissions by blood inoculation. The needle experiment shows also that an exceedingly small amount of blood is sufficient for transmission of our strain at this stage.

(iii) The choice of vectors out of the large number of blood-sucking insects available must to a certain extent be arbitrary. With cyclical or hereditary transmission, there is usually a considerable degree of specificity, and the selection of the appropriate vector becomes of prime importance. Although cyclical transmission of anaplasmosis by biting flies is extremely unlikely, we considered that some experiments should be undertaken, and we chose *Stomoxys calcitrans,* for this species probably has more opportunities of transmitting blood parasites of cattle than has any other. With mechanical transmission, on the other hand, there is no real specificity, and there is a wide choice among those insects that are structurally adapted both to contaminate a feeding puncture with material obtained at a previous feed and to retain that material unaltered between the feeds. *Stomoxys* and *Tabanus,* both by reason of the structure of their mouth parts and their habits, are eminently suitable for mechanical transmission; and, moreover, we had the work of Sanborn, Stiles, and Moe to guide us, and the subsequently published results of Sanders (1933) lent support to our choice of *S. calcitrans.* The best that can be said of our choice of vectors is that the species we chose seemed on every ground to be more likely to be capable of transmitting anaplasmosis than other blood-sucking Diptera.

(iv) The number of times the flies fed on the recipient calves is shown in the fourth column of Table 1. These figures are quite as large as those used in many successful transmission experiments, and,

when the opportunities for feeding under natural conditions, even when flies are abundant, are considered, they appear to us to be quite adequate to exclude lack of sufficient opportunities of transmission as a possible cause of our failures.

(v) In the mechanical transmission experiments, the intervals between the time the proboscis of the fly was withdrawn from the donor and the time when it actually pierced through the skin of the recipient varied from 9 seconds to 5 hours 51 minutes. If one assumes, as we think one should, that conditions on the proboscis of the flies are not entirely favourable to the parasites, then the shorter the intervals the more likely that transmission will occur. The following figures (Table 3) show the number of bites administered within the half-minute and one-minute periods.

Table 3.

Species.	Experiment.	Time Intervals.	Number of Bites.
S. calcitrans	3	17–30 secs.	45
S. calcitrans	„	31–60 secs.	103
S. calcitrans	4	9–30 secs.	149
S. calcitrans	„	31–60 secs.	88
T. circumdatus	..	10–30 secs.	71
T. circumdatus	..	31–60 secs.	32

Even under the most favourable conditions, with beasts adjacent to one another in stalls and an abundance of flies, *S. calcitrans,* when disturbed from an animal on which it is feeding, usually takes more than half-a-minute to settle down to feed once more. Figures are not available, but we are very doubtful whether *S. calcitrans* would, under the most favourable field or stall conditions, recommence feeding within ten seconds after being interrupted. We have observed that with all Tabanidae the time taken to resume an interrupted feed is much longer than with *Stomoxys.* We consider, therefore, that our experimental intervals were more favourable for mechanical transmission than those that are likely to occur under natural conditions in Australia.

In our cyclical transmission experiments we allowed intervals of from 2 to 42 days, the bites being fairly evenly distributed throughout this period. Judging from what is known of the duration of life of adult *S. calcitrans,* of the decreasing probability of a fly surviving in nature to meet a susceptible recipient as the period of incubation is increased, and of the known times of cyclical development of other blood parasites, the periods allowed would appear to be quite adequate.

(vi) The thickness of the skin on different parts of the body must be considered. In all our experiments the back was used; however, the skin of other parts, the eyelids, the axilla and groin, and the scrotum, is much thinner, and, therefore, possibly more easily penetrated. But

two points have to be considered. In the first place, differences in thickness of the skin on different parts of the body are due not so much to differences in thickness of the epithelium as to differences in the corium. The depth below the surface of the capillaries in the papillae of the corium of a young steer were found on section to be approximately 0·05 mm. in sections of skin taken from the back near the midline, and only slightly less in sections of skin from the belly. Moreover, the flies readily drew blood from the sites we used, and frequently droplets oozed up from the punctures they had made. In the second place, *S. calcitrans* in nature feeds largely on the legs, back, sides, and belly-wall; it is not commonly seen actually on the scrotum, although it is common on the adjacent parts of the belly; and it rarely attacks the axilla or groin. Tabanidae frequent the lower parts of the legs and the belly; they, too, are rarely seen on axilla, groin, or scrotum. Consequently, we do not feel that we can criticise our experiments seriously on the grounds of the sites chosen for administering the bites. Moreover, Stiles (1936) has reported successful transmission with an *intradermal* injection of infected blood.

(vii) During the period, however brief, when the infected blood is on the proboscis of the fly, it encounters chemical and physical conditions that may be unfavourable to the parasite. The chemical conditions are the salivary juices, which we cannot alter, but the physical conditions can be varied to suit the experiment. In view of the fact that parasites can survive considerable periods in blood *in vitro* at temperatures between 42°F. and 100°F., it hardly appears that temperature would affect mechanical transmission. However, our experiments were carried out at temperatures between 46°F. and 98°F., so a considerable variety of conditions likely to occur in nature was covered.

Concentration of the blood by evaporation or actual drying is much more likely to affect the parasites adversely, and consequently the humidity of the atmosphere in which they are working must be seriously considered. A saturated atmosphere would obviously be the best, for it would have no effect on the blood itself, and would exclude the possibility of ill effects due to evaporation. Unfortunately, we have no records of the humidity of the actual atmosphere in which the flies were working, for they were working in tubes under conditions very different from the air of the insectary. There is, however, good reason for believing that the humidity in the tubes approached saturation in most of the experiments, for fine films of moisture were deposited in many of them while they were in contact with the skin.

Generally speaking, temperature was satisfactory for mechanical transmission, and humidity was satisfactory in three out of five experiments, but may possibly have been lower than prevails in the summer in many parts of tropical Australia in the other two. However, none of the other workers with biting flies give any indication of the climate in which they worked, so it is impossible to say whether the discrepancies between their results and ours are due to climate.

With cyclical transmission, conditions are reversed. The blood is taken into the gut of the fly, the variations in relative humidity do not affect it, except in so far as they influence the well-being of the host. Temperature, however, is well known to be of great importance in

influencing the development of all kinds of blood parasites in their intermediate hosts. The temperature at which the flies were kept, except for the relatively short intervals of feeding, ranged from 70°F. to 75°F. Rosenbusch and Gonzalez (1923) state that *Boophilus microplus* only transmits anaplasmosis when the external temperature is high, about 34°C. (93°F.), and attribute their failure in Buenos Aires to transmit the disease with these ticks to the lower temperatures at which they were working there. The temperature of our own warm room may, therefore, not have been sufficiently high for development to occur.

(viii) As a negative control, we kept a healthy calf in the insectary with the donors. This calf was subjected to exactly the same treatment (blood examination, etc.) as the other calves, except that flies were not fed on it. It remained healthy, and was subsequently shown by cross-inoculation to be susceptible and to have remained uninfected. Other healthy calves served incidentally as negative controls from time to time, for we have had no case of accidental infection with *A. marginale* either in the insectary or in the open. Actually, all our transmission experiments but one were negative, so these controls only serve to validate a single positive needle experiment.

(*b*) *Did we effectively prove that we had failed to transmit* A. marginale *by means of biting flies?*

(i) Did we allow sufficiently long incubation periods before performing cross-inoculation? The incubation periods allowed before cross-inoculation in six experiments were: from last bite—80, 84, 90, 95, 105, 158 days; from first bite—89, 103, 109, 122, 122, 161 days. In the two experiments in which cross-inoculation was not performed, the incubation periods to the death of the animal were respectively 74 to 81 days (including 12 days after splenectomy) and 31 to 70 from last and first bite. In the negative needle experiments, periods varying from 51 to 105 days were allowed before performing cross-inoculation. The incubation periods for successful transmission experiments recorded in the literature are set out in Table 4.

TABLE 4.—INCUBATION PERIODS OF *A. marginale*, NATURALLY TRANSMITTED.

Species.	Stage.	Author.	Incubation Period.
(1) *Ticks*—			days
B. annulatus	Larvae ..	Dikmans ..	59
B. decoloratus ..	Larvae ..	Theiler ..	52, 70, 78
Rh. simus	Larvae ..	Theiler ..	75
Rh. bursa	Larvae ..	Brumpt ..	34
Rh. bursa	Larvae ..	Sergent ..	33, 38
Rh. sanguineus ..	Nymphs ..	Rees	24
D. variabilis	Nymphs ..	Rees	32, 35
D. variabilis	Adults ..	Rees	34, 35
D. andersoni	Nymphs ..	Rees	28, 29
D. andersoni	Adults ..	Rees	30, 31
H. lusitanicum ..	Larvae ..	Sergent ..	61

TABLE 4.—INCUBATION PERIODS OF *A. marginale*, NATURALLY TRANSMITTED—*continued.*

Species.	No. of Bites.	Author.	Incubation Period.
(2) *Flies*—			days
Tabanus gracilis *T. sulcifrons* ..	41	Sanborn *et al.* ..	106–113
Chrysops sequax *T. gracilis* .. *T. sulcifrons* .. *T. venustus* .. *T. fuscicostatus* *Silvius pollinosus*	43	Sanborn *et al.* ..	24–38
T. gracilis	79	Sanborn *et al.* ..	30–38
T. sulcifrons	24	Sanborn *et al.* ..	60–66
T. venustus	115	Sanborn *et al.* ..	42–73
T. fumipennis ..	100 (approx.) ..	Sanders ..	62
Stomoxys calcitrans ..	Several hundred	Sanders ..	42

Using mechanical means of transmission, such as lancets and goads, the incubation periods recorded vary between 35 and 47 days; in our positive needle experiment the period was 31 days. Thus, the incubation periods we allowed before performing cross-inoculation were well above the average and frequently above the maximum recorded. We consider that they were adequate. Moreover, the incubation periods in the recipients after inoculation with infected blood were not unusually short. In addition, as will be described below, anaplasmosis is transmissible during the incubation period, and also certain of the experimental animals were splenectomised, which tends to shorten the incubation period.

(ii) Experiments undertaken to determine whether blood is infective when taken during the incubation period were somewhat marred by the very short incubations that chanced to occur in the donors used.

Two calves were given intravenous injections of blood from donors which were respectively at the 7th and 11th day of incubation of *A. marginale* infection. No parasites could be found in the blood of the donors even after prolonged search, but very scanty parasites were detected in both on the following day. Both recipients reacted.

Later, during an investigation into ephemeral fever, we obtained additional evidence of the infectivity of blood during the incubation period of *A. marginale*. Ephemeral fever is a virus disease of cattle, and is characterized by a short incubation period (2 to 10 days, most frequently 3 days) and by the transitory nature of the fever. In order to perpetuate the strain it is necessary to withdraw blood during the fever, i.e., usually on the 2nd or 3rd day after incubation. We used some animals that were carriers of anaplasmosis, but no longer required as donors. Not only did all those animals inoculated directly from carriers duly develop anaplasmosis, but also animals sub-inoculated from them while they were suffering from ephemeral fever, i.e., during

the first few days of incubation of *A. marginale*. The second sub-inoculations also produced anaplasmosis, but not the third. Some of the data collected from these records are set out below in Table 5.

TABLE 5.—RESULTS OF INOCULATING WITH BLOOD TAKEN EARLY IN THE INCUBATION PERIOD OF *A. marginale*.

Donor.	Incubation Period of Donor.	Day of Incubation when Blood Withdrawn from Donor.	Recipient.	Dose.	Time *in vitro*.	Result.	Incubation of Recipient.
	days			ml.	days		days
269	*	Second	272	20	0	+	(?) 24
272	(?) 24	Second	277	20	0	+	(?) 34
277	(?) 34	Second	283	20	1	—	..
267	15	Second	274	170	3	+	(?) 20
274	(?) 20	Third	286	230	4	+	29
265	26	Third	246	200	2	+	(?) 30
246	(?) 30	Fourth	281	175	2	+	(?) 34
281	(?) 34	Second	268	20	0	—	..

* No. 269 died of ephemeral fever. It was a locally-bred steer and almost certainly had never had anaplasmosis. All similarly bred animals, which we have used, have proved susceptible to anaplasmosis.

It will be seen from these figures that blood may be infective as early as the second day of incubation.

In some instances the exact incubation period is not known, as the blood was only examined at infrequent intervals until the possibility of transmission so early in the incubation period was realized. In these, the probable incubation period (indicated by a question-mark) is estimated from the stage the infection had reached when it was first discovered.

The results are set out graphically in Fig. 1.

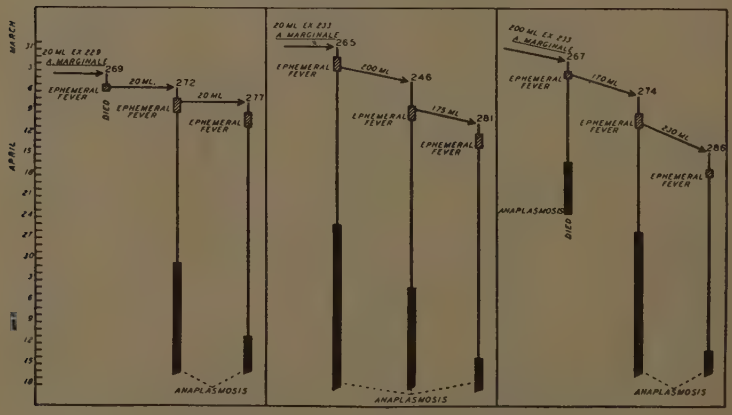

FIG. 1.—Transmission of *A. marginale* with blood taken early in the incubation period.

These results strongly suggest that, had our experimental animals been developing anaplasmosis at the time of cross-inoculation, the recipients of their blood should have developed the disease.

(iii) Splenectomy is a very useful adjunct to this type of work, because the susceptibility of splenectomized animals can be assured, the incubation period is shortened, and latent infections promptly relapse. We are now inclined to believe that splenectomized animals should always be used for this type of work. Three of the recipients in the fly transmission experiments were subsequently splenectomized. In Experiment 3 with *S. calcitrans,* cross-inoculation was performed 53 days after splenectomy, and in the experiment with *T. circumdatus* it was performed 28 days after splenectomy. In both these experiments we can, therefore, be satisfied that the animals neither harboured a latent infection nor were undergoing a prolonged incubation. In Experiment 2 with *S. calcitrans,* cross-inoculation was not performed, and the animal survived only 12 days after the splenectomy. Even this short period is, however, suggestive, for we have found in our splenectomized animals that the average period for relapse or incubation is about 8½ days.

(iv) Cross-inoculation, provided a sufficient interval is left before performing it, remains the ultimate criterion both of the susceptibility of the experimental animal and the absence of a latent infection. We performed cross-inoculation in six out of the eight fly experiments, and in five of the eight needle experiments in which no infection appeared, and we have given reasons above for considering that the pre-inoculation periods allowed were adequate. Of the two fly experiments in which cross-inoculation was not done, the recipient in Experiment 2 survived 12 days after splenectomy, and in Experiment 7 the recipient's blood was injected into a clean calf, No. 212, after an incubation period of 31-70 days. In three of the needle experiments the experimental animals were merely given virulent blood and duly developed the disease. Their blood was not tested for latent infection.

(c) Conclusions.

We can be reasonably certain that we did not transmit *A. marginale* under our experimental conditions by the bites of *Stomoxys calcitrans* or *Tabanus circumdatus.* These conditions varied from very short mechanical to long cyclical transmission, and included a fair range of climatic conditions. They were sufficient to exclude abundance of flies, or accessibility of hosts to the flies, as influencing the results, and were sufficient also to throw serious doubt on the possibility of biting flies transmitting anaplasmosis in this district. This view is supported by the fact that our experimental animals have since been released from insectary quarantine, and no natural cases have occurred, although carriers and susceptible animals are running together and various blood-sucking Diptera are not scarce. We cannot say with the same assurance that transmission by *Stomoxys* or Tabanids would not be likely to occur in North Queensland.

The needle experiments support the other observation, and suggest two reasons why the flies failed to transmit *A. marginale.* In the first place, we succeeded in one experiment out of the three with quick, deep punctures, but failed consistently with quick, shallow punctures under similar conditions of interval, climate, etc. Although blood appeared at the shallow puncture, just as at the deep ones, no doubt the dose of infected blood delivered to the recipient would be influenced by the depth of puncture, and Taylor (1935) has shown that there

is a lower limit to the quantity of blood that is infective. Secondly, we succeeded once with quick, deep punctures, but failed with delayed, deep punctures, although the delay was only 10 seconds. Conditions on the point of a clean hypodermic needle were clearly inimical to the anaplasms, which suggests that they may not be altogether favourable on the proboscis of a blood-sucking fly.

Why Taylor in England, Morris and his co-workers in Montana, and ourselves in Australia failed with biting flies, while the American workers in Oklahoma and Florida succeeded apparently with ease, is not clear, and there is insufficient detail in the published results to permit of a critical analysis. There is, on the one hand, however, a distinct hint in the general literature that the American strain of *A. marginale* may be more virulent and more highly infective than the African or Australian strains, and this may conceivably explain the differences in the results obtained. On the other hand, climatic differences may also be of importance. It is, at any rate, interesting that the positive experiments were carried out in Oklahoma and Florida, which lie south of Lat. 37°N. and Lat. 31°N. respectively, while the negative findings were recorded in Montana, which lies north of Lat. 45°N., and in the south of England, Lat. 51°N. In the southern hemisphere, suspicious accidental infections occurred at Townsville, in the tropics, but only negative results were obtained at Canberra, Lat. 35°S.

7. Acknowledgments.

We were indebted to Dr. J. A. Gilruth for suggesting the work, for arranging for the introduction of *A. centrale,* and for vigorous and stimulating discussion; we also owe a debt of gratitude to Dr. John Legg for supplying infected blood and demonstrating the operation of splenectomy; and to our colleagues, the late Miss M. Fuller and Messrs. W. James and T. G. Campbell, who gave much help in collecting and rearing flies and in the tedious work of feeding them on the cattle. Mr. James also prepared the figure.

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A Note on the Possible Anthelmintic Value for Sheep of Phenothiazine Incorporated in Feed or Lick.

*By H. McL. Gordon, B.V.Sc.**

Phenothiazine has a high anthelmintic value against a number of the important worm parasites of sheep, it prevents the development of larvae in the faeces of treated sheep, and it has a relatively low toxicity for sheep. An investigation of the effects of phenothiazine when taken by sheep in small repeated doses with supplementary food or lick therefore seemed to be justified.

The investigation is not yet completed, but the many enquiries that have been received concerning the possibility of administering phenothiazine in salt licks or feed make desirable a brief record of the results already obtained in this laboratory.

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Preliminary experiments showed that daily doses for five days of 1 g. or 2 g. phenothiazine to weaners and adult sheep in pens were effective against *Haemonchus contortus* and *Oesophagostomum columbianum,* but not against *Trichostrongylus* spp.

The mean daily intake of 0·4 g. to 1·4 g. of phenothiazine in a food supplement by weaners in pens during a period of fifteen days, caused fairly good reduction in the number of *H. contortus* developing from larvae administered concurrently, but a similar effect was not obtained when *Oe. columbianum* larvae were administered.

When sheep consumed adequate amounts of lick (sheep in pens) or food supplement (field trial) containing phenothiazine the anthelmintic effect against *H. contortus* and *Oe. columbianum* was satisfactory. However, the amount of phenothiazine ingested in this way exerted but slight anthelmintic effect against *Trichostrongylus* spp.

Several of the sheep irregularly ingested only small quantities of the licks or foods containing phenothiazine. In these cases there was temporary but great reduction in the number of larvae developing as well as reduction in the number of eggs in the faeces, but no apparent reduction in the number of worms present in the gut. These results would temporarily reduce contamination of the pastures, but give no relief of the parasitism of the sheep.

In a field trial, 90 per cent. of 36 sheep consumed sufficient of a food supplement containing phenothiazine to give satisfactory efficiency against *H. contortus;* 80 per cent. of them consumed sufficient to give a satisfactory effect against *Oe. columbianum;* only 25 per cent. consumed enough for satisfactory efficiency against *Trichostrongylus* spp. The total quantity of phenothiazine consumed was equivalent to two-thirds of one full dose per sheep during one period of six days, and a little more than one full dose per sheep during a second period of six days. Some individuals consumed considerably more than their share, and others thus obtained correspondingly less of the drug. If the same quantity of phenothiazine had been administered to the sheep in a single dose as a drench its anthelmintic effect would certainly have been greater. Moreover, drenching would have ensured a proper dose for each sheep, and a more uniform anthelmintic effect, including action against *Trichostrongylus* spp.

Therefore, although further work is required and will be undertaken, the administration of phenothiazine in salt licks or in a food supplement cannot be recommended at present, and the reasons may be summarized as follows:—

1. The amounts of lick or supplement consumed by the sheep are irregular and the anthelmintic effect varies accordingly.

2. A full dose of phenothiazine consumed gradually in lick or feed over a period of several days has little effect on *Trichostrongylus* spp.

3. To use phenothiazine in these ways is uneconomical, because better results can be obtained by administering the same quantity of the drug in a single dose as a drench.

Reports from the United States of America indicate that continued use of phenothiazine in salt licks resulted in serious discolouration of the fleece, which could not be removed by scouring. This has not been our experience in Australia, but further observations are needed.

The Preparation and Examination of Faecal Cultures for the Differentiation of Larvae of Sheep Nematodes.

*By H. V. Whitlock.**

Summary.

Techniques for the preparation of larval cultures from sheep faeces and examination of larvae recovered therefrom are described. Improvements of the technique formerly used are described, including (*a*) mechanical mixing of faeces, (*b*) a trough-slide for holding culture washings during microscopical examination, and (*c*) the use of iodine solution for killing and staining larvae.

1. Introduction.

The technique formerly used at this laboratory for the preparation of larval cultures from sheep faeces and the recovery of the larvae for diagnostic purposes, had its origin in the work of Veglia (1915). The methods described by this author have been modified in various ways, but the principles dictated by the reactions of larvae to heat, moisture, light, and fermentation have remained essentially the same.

Both the older and the improved techniques are described and their advantages and disadvantages are discussed.

2. The Older Technique.

Faecal cultures are prepared by crumbling fresh faeces or breaking them down with an old scalpel in a glass jar of 2 oz. capacity fitted with an aluminium screw top. When the faeces are soft or diarrhoeic the consistence is brought to that of normal sheep faeces by the addition of animal charcoal or helminthologically sterile sheep faeces. The jar is half-filled, care being taken to avoid solid packing and to maintain a spongy texture permitting adequate aeration. Water is then allowed to drip into the culture, particular care being taken to ensure wetting of the edges of the faecal mass. The amount of water required is judged by experience—too much results in putrefaction, too little in desiccation. The lid is then screwed on firmly, but the jar is not made airtight. The culture is incubated at 27°C. for seven days during which time several examinations are made to detect and correct any desiccation or putrefaction. The faecal mass should be moist and after a day in the incubator droplets of water of condensation should appear on the walls of the culture jar. By means of this moisture the infective larvae ascend from the faecal mass and can then be recovered free from faecal material.

After incubation the culture is prepared for examination by exposure to mild diffused daylight for about 30 minutes.

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The third-stage, infective larvae migrate upwards on the clean sides of the jar and are collected in the manner shown in Fig. 1. The jar is held, neck downwards, at an angle of about 30°, water is added from a pipette and the jar is rotated to collect the larvae from the walls. By keeping the jar at an angle the water is prevented from running back into the faecal mass.

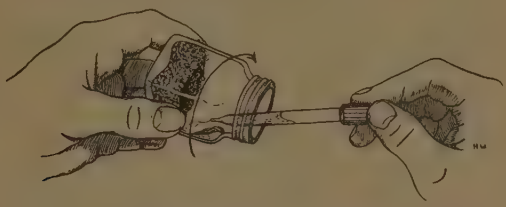

FIG. 1.—Method of washing larvae from cultures.

The water containing the larvae is then run on to a glass microscope slide and the larvae are killed by passing the slide over a Bunsen flame several times. Larvae can then be differentiated by microscopic examination using magnifications of 40 and 100.

The disadvantages of this technique are that—

(*a*) much time is consumed in breaking down faeces for preparation of cultures;

(*b*) in killing the larvae by heat there is a risk of cracking the slides and losing the larvae;

(*c*) the area of a slide limits the amount of fluid which can be used to wash larvae from the sides of the culture jar, whereas an adequate amount of water is necessary to ensure collection of all the larvae present—this is specially important when comparatively few larvae are present;

(*d*) there is a risk of the suspension of larvae being spilled from the slide on to the microscope stage with consequent loss of many larvae and delay through the need to dry the stage before proceeding with the examination.

3. The Improved Technique.

The preparation of the faecal cultures is made in essentially the same way as for the older technique, but the breaking down of faeces and the incorporation of helminthologically sterile sheep faeces when necessary is carried out by means of the electric mixing device described by Kauzal and Gordon (1941).

The most satisfactory medium for converting soft or diarrhoeic samples to a consistence suitable for culturing is dried, finely ground, helminthologically sterile sheep faeces. This medium is prepared by drying and sterilizing faeces on trays in an oven at about 200°C. for 120 minutes. The dry faeces are then ground to a coarse powder in a mill.

Larvae are examined in a specially constructed trough slide (see Fig. 2) with four compartments about 20 mm. by 15 mm. and 3 mm. deep to contain about 1 ml. water each.

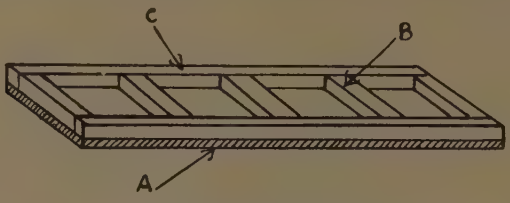

FIG. 2.—Special Trough Slide—
A. Glass Microscope Slide.
B. Celluloid or Glass Cross Pieces.
C. Celluloid or Glass Edge Strips.

The walls of the troughs are made of glass or celluloid strips on a glass microscope slide. The troughs should not be less than 3 mm. deep. Acetone is used for joining celluloid strips, and Canada balsam for attaching celluloid to the glass base. Celluloid bases are unsatisfactory owing to warping and progressive opacity.

Larvae are washed from cultures into the trough and washings from four cultures can be examined on a single slide. Adequate washing of the walls of the culture jar can be achieved by using a larger amount of water than was possible with the technique formerly in use. Larvae are killed by adding the washings to a few drops of aqueous iodine solution placed in each trough. The solution is prepared by diluting a saturated solution of iodine and potassium iodide with an equal quantity of water. The larvae are stained a light brown by the iodine and this aids recognition of differential features.

This technique overcomes all the major disadvantages of the former method, and greatly reduces the time required to deal with large numbers of faecal samples from sheep in field trials. By using the egg-counting technique described by Gordon and Whitlock (1939), the modifications described by Kauzal and Gordon (1941), and the methods described in this paper, two laboratory technicians can complete egg counts and the preparation of faecal cultures from 100 sheep in about 2½ hours. One technician can make a differential count of larvae from 100 cultures in about 2½ hours.

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The Use of Mineral Oils and Tar Oils for Wheat Weevil Control.

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Summary.

Factors affecting the kill obtained in tests of oil emulsions as contact insecticides against wheat weevils are discussed.

A mineral oil emulsion with an added toxicant has shown great promise in tests. "Unfortified" mineral oil emulsions have limited application in practice for treating infested grain sheds, stacking sites, &c. Creosote emulsions of suitable type, however, should prove effective if used at a sufficiently high concentration.

For treating infested cornsacks, tar-oil emulsions are more reliable than mineral oil emulsions.

Results of experiments with straight (unemulsified) oils are discussed. A 1-2 per cent. solution of dinitro-o-cyclohexylphenol proved a very effective contact spray.

1. General.

Where wheat is handled and stored in bags, clean grain is liable to become infested by weevils which carry over between seasons in the timberwork of sheds, the surface and mouse-proof fencing of stacking sites, stack dunnage, &c. Owing to the difficulty and expense of dealing effectively with weevil infestation once it has become well established in stacks of bagged wheat, the sterilization of storage premises, before fresh grain is moved into them, must be regarded as a matter of considerable importance. This aspect of the general problem of wheat weevil control has been investigated at Canberra. At a later date it is hoped to publish a detailed account of the work, which is still proceeding. However, as it has been necessary to make practical recommendations regarding methods and materials before the various lines of investigation had been properly completed, and as a number of points of interest and importance have already been established, it was felt that a brief statement might be issued with advantage at this stage.

The obvious materials to select for stack-site, shed, and dunnage sterilization are those that kill weevils by contact action. If a material has some fumigating effect in addition to contact toxicity, so much the better: by creating a toxic atmosphere in crevices, &c., where weevils harbour and are likely to find protection from direct contact with the applied fluid, the efficacy of the treatment would be increased. Stomach toxicity in a material is unlikely to be important, though it might play a minor part by rendering spilled wheat unsuitable as a food and breeding ground for weevils.

For general shed and stack-site treatment, for which very large quantities of material would be required, only cheap and easily obtainable materials can be seriously considered. On the score of cheapness and availability, mineral (petroleum) oils and creosotes (unfractionated tar oils) stand alone. The contact-insecticidal value of mineral and tar oils, applied as emulsions, has been established in horticultural practice, and attention was concentrated chiefly on these materials.

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2. Methods of Testing.

Besides ourselves, entomologists in one or two of the wheat-producing States have given attention to oil emulsions. The results obtained at the different centres have, to some extent, been contradictory. Although they have for the most part remained unpublished, they have become known in interested circles. The investigations at Canberra were therefore intensified in the hope that a better understanding of the factors affecting the efficacy of oil emulsions against wheat weevils would provide an explanation of these contradictions. The fact that this hope has been very largely realized provides an additional reason for issuing some statement at the present time; for unexplained contradictions in research results constitute an embarrassment as well as a challenge.

It was found that the kill obtained with an oil emulsion could vary over a wide range according to the technique employed for testing, without any clue being provided by the behaviour of the controls. The explanation of this, of course, provides the key to the puzzle of the contradictory results that have been obtained. Before discussing the experimental data which provide at any rate a partial explanation of these widely varying kills, it seems desirable to draw attention to an important point in connection with the testing of materials intended for sterilizing sheds and stacking sites.

In the development of insecticides for special purposes, the final stage in the experimental work will normally consist of large-scale field trials. In the case of contact insecticides for use against wheat weevils, however, it is virtually impossible to carry out what might be termed practical field trials that would have any real value. One cannot treat premises and judge the efficiency of the treatment from the degree of infestation subsequently developing in the grain stored there. Some quantitative measure of the kill obtained is more or less essential. Most entomologists who have inspected empty grain sheds and stacking sites, and searched for the largely invisible infestation, would probably concur in our opinion that a mere examination, however carefully carried out, is unlikely to provide a sufficiently reliable indication of the effect of a treatment. If, immediately after treatment, batches of insects are collected, or confined in some way on the spot, for determination of the mortality after a suitable interval, an artificial factor is introduced which renders the trial of little if any greater practical value than small-scale tests carried out under controlled conditions. In any case, as conveniently located, empty, infested sheds or stacking sites are usually not available when required, the assessment of materials developed for their treatment must be based on small-scale tests.

In addition to the obvious factors such as the type of oil used, the concentration, and the dosage, the percentage kill obtained in a test of a mineral or tar oil emulsion will depend on the following:—(1) the age of the insects used; (2) the method of treatment, including—where a spray technique is employed—the nature of the surface or substratum on which the insects are exposed; (3) the conditions to which the insects are subjected in the interval between treatment and the mortality count, carried out a day or two later; and (4) the nature of the emulsion.

(a) The Age of Test Insects.

In common with other workers, we use the rice weevil (*Calandra oryzae*) in our routine tests, since it possesses a markedly higher resistance to contact insecticides than the other two primary pest species, *Calandra granaria* and *Rhizopertha dominica*. *Tribolium castaneum*—a secondary pest of grain, but a major pest of flour mills—has been used in some experiments, because the materials used for stack-site sterilization will find a useful application for treating flour stores and mill premises generally. This insect in some tests appeared to be more, and in others less, resistant than *C. oryzae*.

When insects bred under standardized conditions are used in tests and large numbers of them are required, as has been the case at Canberra, it simplifies matters considerably to use young individuals. In our tests of oil emulsions we have made a practice of using *Calandra oryzae* that have emerged from grain during the previous week, for brevity termed 0–1 week old. (Actually, this age group, sieved from cultures on Monday, may by the end of the week contain individuals that emerged up to 11–12 days previously.) *C. oryzae* has an adult life of several months. At first we feared that the young individuals used in our tests might be more susceptible to contact insecticides than were older insects. However, comparison between 0–1 week old laboratory bred insects and insects selected at random from a natural mixed-age population revealed that the former were consistently and often markedly more resistant.

In two instances only have 0–1 week and mixed-age weevils been used in the same experiment. In the first a mineral oil emulsion was used, employing, for comparative purposes, two somewhat different techniques. With one technique the kills of 0–1 week and mixed-age insects were respectively 1 per cent. and 18 per cent., with the other the figures were 61 per cent. and 70 per cent. The second experiment was a spraying test with a creosote. The two batches of insects were sprayed one after another from the same lot of emulsion, receiving identical dosages and treatment. The kill in the 0–1 week weevils was 4 per cent., in the mixed-age 71 per cent. More than half-a-dozen emulsions, some of mineral and some of tar oils, have been tested against 0–1 week and mixed-age weevils in separate experiments, but using the same treatment, the same concentration, and the same dosage. Comparison of the kills obtained indicates a consistently higher resistance of the young stock, the difference in susceptibility being, in one or two instances, as great as that revealed in the experiment with creosote just mentioned.

In one isolated experiment, using a creosote emulsion as a dip, and laboratory-bred insects only, 0–1 week old *C. oryzae* showed a *lower* resistance than insects of 1–2 and 2–4 week age groups. This suggests that the previously mentioned difference in susceptibility might be due rather to differences in physiological condition than to mere age, since the mixed-age weevils were obtained from a population which probably had not enjoyed such favourable conditions as had the laboratory-bred insects. It is intended to check this point in further experiments. It might be mentioned, however, that the results of another series of experiments indicate that age is probably the most important factor in determining relative susceptibility. In these experiments straight (unemulsified) oils were applied with an atomizing spray gun.

Tribolium castaneum 1–2 weeks, 2–3 weeks, and 5–7 weeks old were used, all the insects having been bred under standardized conditions in the laboratory. In no one experiment were insects of more than one age group employed; but comparison of the results of different experiments seems to provide definite indication of a consistent and very marked increase in susceptibility with increasing age.

From the information set out in the preceding paragraphs two conclusions can be drawn:—First, that a comparison between the results of tests in which insects of different ages and bred under different conditions were used is liable to be misleading; and second, that for the assessment of the relative efficacy of contact insecticides it is desirable to use young, laboratory-bred weevils, the test being made more rigorous thereby.

(b) Method of Application and "After-Treatment."

It is convenient to discuss these two factors under one heading, as it is not always possible to draw a hard-and-fast distinction between them.

For the assessment of the contact toxicity of insecticides, either a spray or a dip technique can be used; and it is recognized that between the actual application of the material and the mortality count the test insects should be subjected to conditions ("after-treatment") which do not in themselves tend to cause mortality or prevent recovery of the treated insects. In the early stages of our work with emulsions we employed, almost exclusively, a five-minute dip (complete immersion), then placing the insects, after being allowed to drain for a few seconds on wire gauze, in small jars containing wheat, which were kept in a constant temperature and humidity room until the mortality count, carried out at least 24 hours later.

At first we were inclined to believe that an emulsion which, in laboratory tests, had consistently given kills of close on 100 per cent. could be relied on to be effective when sprayed on to weevils in such a way as to wet them thoroughly; and conversely that an emulsion which failed to give high kills in dip tests would not be effective in practice. We were mistaken in these assumptions, not realizing the importance, in the first instance, of the varying absorptive powers of the surfaces on which weevils might have to be treated, and in the second, of the slow-acting toxicity of certain emulsions. Although dipping tests furnish a reliable indication of what may be termed the immediate contact toxicity of an emulsion, they do not reveal the full value of materials such as tar oils, which seem to possess a fumigating action that requires a fairly long period to show its effect.

When these facts were appreciated, the dipping tests were augmented, and largely superseded, by spraying tests. A small orchard spray, the reservoir of which can be connected with a compressed air pump, was set up so that the cone of liquid issuing from the perforated disc nozzle is directed on to a turntable rotated by a small electric motor. The large glass petrie dishes, in which the test insects are exposed, are placed on the turntable, the rotation of which counteracts any unevenness in the spray-cone and facilitates the timing of the application. The test insects can be exposed in the dishes on surfaces with varying absorptive powers, from a layer of sand deep enough to absorb, completely and almost instantaneously, the applied liquid, to a single sheet of blotting

paper which is insufficient to absorb, completely, the smallest dose used in our tests (approximately equivalent to an application of one gallon to fifty square feet). Intermediate degrees of absorption can be obtained by varying the depth of the sand layer, or using more than one sheet of blotting paper. After the application of the spray, the dishes are covered with organdie, which prevents the escape of the weevils without affecting evaporation or the diffusion of vapour. Various after-treatments can be employed. The insects can be left on the surface on which they were sprayed until the mortality count is made, after any desired interval. They can be subjected, in the dishes, to varying conditions, e.g., of air movement or humidity, likely to occur in practice. They can be removed after varying intervals, and placed on dry sand or in jars of wheat (representing, in practice, escape from the treated area), and untreated weevils can be placed on the sprayed sand or paper (representing the wandering, on to a treated area, of insects that have escaped direct contact with the insecticide).

It should not be difficult, using this adaptable spraying technique, to determine in the laboratory how any oil emulsion is likely to behave in practice under the varying conditions that are known, or suspected, to affect its toxicity to weevils. On the results of experiments that have already been carried out, it is possible to make the following statements with a reasonable degree of assurance:—

(1) Emulsions which have a high degree of contact toxicity, as indicated by dipping tests, but which lack chemical toxicity, will only be effective when used as sprays *if the surface can be saturated to such an extent that some surplus fluid will be left unabsorbed for a time.* (Mineral oil emulsions without added toxicants fall into this category. Their toxicity against insects is generally regarded as due to physical rather than chemical action. "Chemical toxicity" includes the presumed fumigant action—see below—of tar oils.)

(2) If the surface remains saturated for a considerable time—say the best part of a day—*and the weevils remain on the treated surface,* even emulsions with poor contact toxicity, as measured by a dip test, may give high kills. But if the weevils can escape from the treated surface after a relatively short interval, the kill with these emulsions (including those with chemical toxicity) will fall; in fact, under these conditions an emulsion will give a kill of the same general order as it would when tested by the laboratory dipping technique. It might perhaps be mentioned that observation of outdoor spraying tests carried out at Canberra indicates that although weevils may be temporarily immobilised by the application of an oil spray, they will soon—some almost at once—regain their normal activity and start to wander; though how long this activity lasts, and whether it is regained with all types of spray, we are not in a position to state.

(3) The slow-killing action, which augments the (often poor) "immediate contact toxicity" of tar oil emulsions, seems to be due to a true fumigant effect which, however, only operates over a very short distance, so that the weevils, to be affected, have to remain practically in contact with the liquid. That this effect is due to a toxic vapour is indicated by the following facts:—(i) The kill of weevils, sprayed with a creosote emulsion and left on the sprayed sand, falls sharply if they are subjected to an air current; (ii) if untreated weevils are

placed and left on sprayed sand, the kill obtained will be almost as high as if they had been sprayed on the sand; and (iii) if, before adding untreated weevils, the sprayed sand is covered after a minute or two with a very thin layer of dry sand, to prevent the insects coming into actual contact with the wet surface, a kill of the same order as in (ii) above will be obtained. (A series of experiments carried out by one of us—J.S.F.—with weevils in closed containers demonstrated that creosote vapour possesses a surprisingly high toxicity, and also that the fumigating effect was more marked, as one would expect, in low-boiling-range creosotes.)

(c) The Nature of the Emulsion.

The results of our dipping experiments indicated that the degree of fineness and the stability of mineral oil emulsions were of greater importance, in determining their contact toxicity to weevils, than the type of oil used. We only obtained consistent kills of, or near, 100 per cent. with very coarse emulsions that were not far from the limit of their stability. With emulsions of this type, kills of 98 to 100 per cent. have been obtained with oils of widely varying degrees of refinement, and with viscosities of as little as 35 and as much as 200 to 250 seconds Saybolt at 100°F.

The stability and droplet size of an emulsion are inter-related. While we know of no simple, quick, and reliable method of assessing the former, the size of the droplets can be determined in a minute or two by microscopic examination. Except when hard water—which affects the emulsion stability—has been used for diluting the stock, or a chemical toxicant has been added, no mineral oil emulsion has proved effective in our dipping tests unless the diameters of the droplets ranged up to and over (usually well over) 60 microns. An emulsion in which such a range is only attained because of the presence of a few large droplets, with the bulk of the droplets small (say under 10 microns in diameter), will almost always prove ineffective.

In view of the above, it is almost unnecessary to state that no mineral oil emulsion (without added toxicant) prepared from a mayonnaise stock has proved effective in our dipping tests. As even the coarsest mayonnaise stable enough to be handled and stored without danger of "breaking" is unlikely to have droplets of a size approaching that which seems to be necessary, it may be assumed that, in practice, an emulsion of the requisite degree of coarseness can only be prepared from a so-called miscible oil. We refer, of course, only to preparations of the types available commercially. Emulsions with any desired degree of instability or coarseness can be prepared "directly" on a small scale in the laboratory or on a large scale by the tank-mix method. To avoid any possibility of misunderstanding, it might be mentioned here that high kills can be obtained with mineral oil emulsions prepared from mayonnaise stocks under certain conditions, e.g., when weevils are sprayed with them on a relatively unabsorbent surface, and are confined to the treated area.

As has already been mentioned, the degree of hardness of the water used in making up an emulsion will affect its stability and/or droplet size. Furthermore, the droplet size—and with it presumably the stability also—of the dilute "working" emulsion can be altered to a

considerable extent by varying the amount of water used to prepare the "primary" emulsion from a miscible oil, and by varying the vigour of agitation. Using rather extreme methods, we have prepared from the same sample of a miscible oil two emulsions, one coarse and one fine, which gave kills approaching 100 per cent. and zero respectively in dipping experiments.

As the characteristics of an emulsion on which the contact toxicity to weevils very largely depends may be modified to quite a considerable degree by variations in the process of its preparation which can easily escape notice, some check on the nature of the actual emulsion used in a test is obviously desirable. Determination of the range in droplet diameters provides such a check which, though admittedly incomplete, we have found very valuable. Since we adopted it as routine practice the occurrence of puzzling anomalies in our experimental results has been greatly reduced. To cite a concrete example, recently we succeeded—without trying, and without even knowing how the result was achieved—in preparing a very coarse emulsion from a certain miscible oil, although deliberate attempts to produce such an emulsion from this stock had all failed. If the nature of this emulsion had not been revealed in the check of droplet size, the abnormally high kill it gave in a dip test would have remained unexplained, and would have cast doubt on the reliability of our experimental technique.

Probably because tar oil emulsions depend for their efficacy against weevils on something more than "physical" toxicity, their stability and droplet size are not of such paramount importance as seems to be the case where mineral oils are concerned. Nevertheless, these factors are of importance, as is demonstrated by the following results obtained in recent experiments.

(1) From a sample of creosote obtained from a Sydney firm of tar distillers, two emulsions were prepared in the laboratory, each containing 5 per cent. by volume of the oil. In the preparation of emulsion A, 25 per cent. by volume of emulsifier ("Whitcol J") was added to the creosote; for emulsion B, 10 per cent. of emulsifier was used. Emulsion A was more stable and finer than emulsion B, as was indicated by its lesser tendency to cream and by the measured droplet sizes (diameter less than 1.5 microns, as against 3 to 45 microns). In a laboratory dip test the following kills were obtained: with emulsion A, 6 per cent.; with emulsion B, 97 per cent.

(2) Using the same two emulsions applied as a spray at a dosage equivalent to 1 gallon to 50 square feet, the following kills were obtained. Weevils sprayed and left on sand: emulsion A, 28 per cent.; emulsion B, 90 per cent.; untreated weevils placed and left on sprayed sand: emulsion A, 5 per cent.; emulsion B, 92 per cent.; untreated weevils placed and left on sprayed sand over which a thin layer of dry sand had been sprinkled: emulsion A, 9 per cent.; emulsion B, 90 per cent.

The difference in the fumigating effect of the two emulsions, clearly indicated in the second experiment, can be explained on the assumption that the finer and more stable emulsion had no tendency to break when it struck the sand; and the liquid being absorbed into the sand as an unbroken emulsion, the bulk of the creosote was carried too far below the surface for the extremely short-range fumigant action to be effective.

It is clear that in any tests of oil emulsions information should be obtained on the hardness of the water used (Canberra water, incidentally, is soft), and the droplet size should be determined, at any rate where emulsions are made up directly or prepared from a miscible oil. If these data are not obtained and recorded, comparison between the results of different experiments may be valueless, and a reliable assessment of the practical applicability of a test may be impossible.

3. The Practical Application of Mineral (Petroleum) Oil Emulsions.

The position regarding mineral oil emulsions seems to be fairly clear cut. Their application for the general treatment of sheds, stacking sites, dunnage, &c., must be limited because, as our experiments have indicated, they will only give a satisfactory kill where some of the liquid remains, for a time, unabsorbed by the surface to which they are applied. When sprayed or poured on to an absorptive surface such as loose or sandy soil, no reasonable increase in the concentration of the oil, nor of the dosage applied (short of saturating the surface) seems able to insure consistently high kills. It must also be assumed (though this point has not yet been experimentally established) that the immediate run-off would prevent these emulsions from dealing effectively with weevils crawling on vertical surfaces, unless the insects were washed off by the force of the application. It is perhaps important to mention, in this connection, that the spray used in our tests was operated at a low pressure, i.e., 30 lb./sq. in. It is possible that when applied with a pressure of, say, 200 to 300 lb./sq. in., mineral oil emulsions, when sprayed on to absorbent surfaces, will be more effective than we have found them to be. However, as "weevil sprays" will probably more often than not be applied with simple and inexpensive apparatus, e.g., stirrup pumps and watering cans, the results of our tests should furnish a reliable guide to the probable behaviour of these emulsions in practice.

Mineral oil emulsions could find valuable practical application as dunnage dips, and as sprays for treating the floors of grain sheds, flour stores, and stacking sites, *where these are of concrete, wood, or hard-packed soil.* Such surfaces can be flooded with quite a small amount of liquid, and provide ideal conditions for the use of mineral oil emulsions. Incidentally these emulsions, when conditions are suitable, will generally give satisfactory kills at dilutions of 1:40 and over.

A rather serious weakness of mineral oil emulsions of the type which must be considered for weevil control is their sensitivity to variations in water hardness. Whereas casein emulsifiers, commonly used in the preparation of mayonnaise stocks, can tolerate a reasonable degree of variation in water hardness, the soap emulsifiers used in the commercial preparation of miscible oils cannot. We have found that a miscible oil which, with soft water, gives a coarse unstable emulsion with very high contact toxicity, could not be relied on to emulsify properly with water of more than about 10 degrees of total hardness (British units). Conversely, an oil that gives the right type of emulsion with hard water would, with soft water, almost certainly give too fine and too stable an emulsion to possess adequate contact toxicity. It is, of course, possible to compensate for variations in hardness by artificial softening; but this can scarcely be considered practicable as a general policy, as prior knowledge of the degree of hardness of any water used would be required.

It is clear that the complicating factor of water hardness must affect the practical application of mineral oil emulsions, particularly in South Australia, where the ground water is usually hard and rain water generally at a premium.

The preceding paragraphs in this section all refer to mineral oil emulsions which do not contain any added insecticidal toxicant. Two oil companies have prepared for us samples of mineral oil emulsions in which toxic chemicals have been incorporated. The samples submitted by the Shell Company have given very satisfactory results in our tests to date. Being mayonnaise stocks, they should not be affected by reasonable variation in the hardness of the water used for their dilution. They seem to combine the high immediate contact toxicity of the best "unfortified" mineral oil emulsions with the virtues possessed by tar oil emulsions. The cheaper of the two is prepared from a low-viscosity fuel oil, and has given the following results in tests: (1) in a dip test, used at 1:40 dilution, 99 per cent. kill; (2) in a spray test, used at 1:20 dilution, applied to sand, 100 per cent. kill of weevils treated and left on sand using dosages equivalent to 1 and 2 gallons per 50 sq. ft., and also of untreated weevils placed and left on sand sprayed with the higher of the two dosages. Although, as in the case of tar oil emulsions, the kill is reduced if the weevils are subjected to an air current, or are removed and placed on dry sand, this preparation definitely seems to mark an advance on any commercial emulsion we have hitherto tested; and it is not improbable that by the time this paper appears in print the results of further tests will have indicated that this product can be recommended for general use.

4. The Practical Application of Tar Oil Emulsions.

The only tar distillation products cheap enough to be considered for large-scale shed and stack site treatment are creosotes, which are unfractionated tar oils distilling over a wide range—usually about 200° to 400°C. The products marketed by tar distillers to conform to the standards set for wood-preserving creosotes will contain most of the high-boiling oils which our experiments have shown to be more toxic to weevils, as contact insecticides, than the medium and low boiling fractions. However, the latter contribute to the fumigating effect of a creosote, which is of very definite practical value; and therefore the special high-boiling-range creosotes marketed by some firms are probably no more suitable for the purpose in view than are the "standard" lines.

Creosote emulsions are prepared commercially as mayonnaise stocks, usually with casein emulsifiers. Creosote is not emulsified commercially to any great extent, and we have not been able to experiment with a great variety of samples. From our dipping and spraying tests the following points of practical significance have emerged:—

(1) Creosote emulsions tend to be erratic in their immediate contact toxicity—no commercial* emulsion has given, at 1:40 dilution, consistently high kills in our dip tests. At this strength, in fact, most commercial preparations evinced rather poor contact toxicity.

* The term "commercial", used here and elsewhere in this paper, means that the sample in question has been prepared by commercial methods, not that the preparation is available on the market.

(2) Good results cannot be expected from a fine and stable emulsion. This conclusion is based, not only on the results of the experiments with laboratory-made emulsions already referred to, but on our tests of commercial samples. (As in the case of mineral oil emulsions, however, high kills may be obtained *under certain conditions* with emulsions of this type.)

(3) Other things being equal, the efficacy of a creosote emulsion increases steadily with the concentration of the oil, a relation which does not always seem to hold with mineral oil emulsions.

(4) Even with the most effective of the commercial samples, used at 1:20 dilution, consistent kills of 90 to 100 per cent. of weevils sprayed on sand were only obtained with a dosage equivalent to an application of 2 gallons per 50 square feet.

(5) As has already been mentioned, the kill obtained with a creosote emulsion is reduced by air movement.

It would be a great convenience, when faced with the problem of sterilizing premises that provide a wide variety of treatment conditions, to be able to use one material to cope with them all. As we have already indicated, an "unfortified" mineral oil emulsion cannot be relied on to do this; *a creosote emulsion of the right type probably could.* Whether it would do it as effectively and economically as a "fortified" mineral oil emulsion has yet to be determined. The latter, incidentally, suffers from one disadvantage which may prove very serious—the principal constituents are imported.

It would probably be unwise to apply even the most effective creosote emulsion at a dilution of more than 1:20. The bulk-handling authorities in Western Australia apply their preparation—which is as effective as any we have tested*—at a dilution of 1:15 when using it as a spray; and this seems a good lead to follow. It would probably pay to use a more concentrated emulsion still for treating structures such as mouse-proof fences, which are not only notorious harbours for weevils, but are also difficult to deal with effectively in that they are exposed to the wind, and abut on ground which would normally not be treated, and on which weevils could find refuge.

The danger that weevils will escape from a treated area—and thus from the fumigating effect of the creosote—and recover, is unlikely to be serious in practice, for when a whole shed or stacking site is soaked with creosote it will be a very fortunate insect whose chance wanderings take it to a safe spot. The danger of wind reducing the efficacy of a treatment cannot, however, be dismissed as negligible. The air current used in our tests was equivalent to little more than a gentle breeze, and it produced a startling reduction in the kills obtained with creosote emulsions. We consider that it would be wise policy if the treatment of open sheds and sites were carried out, as far as possible, only in calm weather or late in the day. If there is much air movement, the dosage and/or concentration of the emulsion used should be increased.

We are rather doubtful whether the fumigating effect of creosote emulsions used at a dilution of 1:40 would be sufficient to counterbalance their unreliable immediate contact toxicity; therefore we are not prepared to hazard an opinion as to their superiority over mineral oil emulsions as dunnage dips.

* A sample with a modified formula, obtained more recently, was less effective.

Our experience with creosote emulsions indicates that they are liable to vary inexplicably in their effectiveness, and we consider that the only safe policy would be to test every batch before putting it to use. For this purpose a simple dip test should suffice; the creosote emulsions which gave the best kills in our dip tests also performed well as sprays.

5. Oil Emulsions for Dipping Cornsacks.

The sterilization of used bags in which grain is delivered by growers to bulk-wheat receiving centres presents a problem of its own, which is best considered apart from the other practical applications of oil emulsions.

For killing weevils harbouring in cornsacks, emulsions of tar distillation products are more reliable than mineral oil emulsions. The use of the latter is complicated by the fact that coarse unstable emulsions with high immediate contact toxicity break down when bags are dipped in them, the larger droplets forming a coating of oil on the outside of the bags. Thus, as bag after bag is immersed, the emulsion becomes progressively less effective, and the kill of the weevils in the bags drops. Certain moderately fine and stable mineral oil emulsions will, without showing any significant deterioration when a series of bags are immersed in them, give high kills of weevils if the treated bags are stacked wet for a period of about 48 hours before being hung up to dry in the sun and wind. The same factors are probably operating under these conditions as when high kills are obtained in spraying tests with emulsions possessing only moderate immediate contact toxicity—i.e., when the weevils are confined for a fairly long period on the sprayed and saturated surface. On the basis of our experimental results, we recommended two proprietary mineral oil emulsions (actually marketed as horticultural sprays) for use as bag dips. The kills we obtained with them, however, were not quite as consistent nor as high as those obtained with emulsions of tar distillation products; and we consider that mineral oil emulsions should be superseded by the latter if the treatment of used bags is made compulsory in the Eastern States, as it already is in Western Australia.

From our experience with these products in bag-dip tests, we arrived at the conclusion that a suitable tar oil emulsion, used at a dilution of 1:40, can be guaranteed to give virtually 100 per cent. sterilization of infested cornsacks if it is given adequate opportunity to manifest its fumigation effect. This is provided by stacking the freshly-dipped bags in a pile for 48 hours, covering them with a tarpaulin if only a small number are treated. High kills can sometimes be obtained when bags are hung up to dry at once, but in Canberra they seem to be exceptional, and can probably be explained by the climatic conditions occurring at the time. The value of the "wet-stack" technique is indicated by the results of an experiment with a Western Australian creosote emulsion, which gave 100 per cent. kill in wet-stacked bags, and only 4 per cent. in a bag hung up to dry at once. The practice of wet-stacking for 48 hours adds very little to the work involved in treating bags; and as it seems to ensure success, there hardly seems point in trying to develop an alternative, quick-drying technique.

A tar oil product that has given consistently excellent results in our tests is naphtha. It is a low-boiling fraction, and can be obtained

in various grades. The "crude" grade, which seems to be even more effective than the "solvent", costs only a few pence per gallon more than creosote.

Unfortunately the coarser and less stable creosote emulsions, which on account of their high immediate contact toxicity are the ones which will naturally be selected for general purpose sprays, show a tendency to be adversely affected by what may be termed the filtering action of the cornsack fabric. This tendency, however, is not nearly as marked as it is in the case of mineral oil emulsions; probably because creosote emulsions do not attain the same degree of coarseness as the latter; and it is just possible that a creosote emulsion which is up to standard as a general spray could be made to do a satisfactory job when used as a bag dip. It would obviously be very much more convenient if one and the same product could be used for either purpose. Incidentally, it is possible that this desired end might be achieved if a "fortified" mineral oil emulsion were used; the Shell product mentioned in a previous section has not yet been tested as a bag dip.

6. The Use of Straight (Unemulsified) Oils.

The main advantage of using emulsions for treating weevil-infested sheds and stacking sites, &c., is that it enables a relatively small amount of oil to be distributed widely and evenly over the surfaces requiring treatment. If suitable equipment in the form of a power spray with the right type of nozzle is available, the use of unemulsified oils might be more economical in cost and labour. The advantages of using mineral and tar oils "straight" are: (1) the cost of emulsification, and the difficulty of obtaining exactly the right type of emulsification, are avoided; (2) the difficulty of obtaining supplies of water, which might be serious in some country centres, is avoided; and (3) treatments can be carried out—e.g., among and around bags of wheat or flour—where it would be inadvisable to use an emulsion, because of the moisture danger.

In our laboratory experiments to determine the relative efficacy of different materials, we used a paint spray gun with an adjustable nozzle, operated at a constant air pressure of 35 lb. sq. in. The insects (*Calandra oryzae* or *Tribolium castaneum*) were exposed on discs of wire gauze, then placed—still on the gauze—in covered petrie dishes until the experiment was completed, after which they were removed and placed in small jars of wheat which were kept in a constant-temperature room until the mortality count, usually carried out four days later.

The use of a nozzle producing what is virtually an atomized spray has distinct disadvantages. Tests have shown that an alteration in nozzle adjustment, giving a slower rate of application and finer droplets, may result in a marked decrease in the kill obtained with the same dosage as measured by the amount of liquid passing through the nozzle—presumably because there is a greater tendency for dispersal in a fine spray. Although we attempt to compensate for differences in the viscosities of the various liquids tested by suitable adjustments of the nozzle, it is recognized that the standardization achieved in our tests is far from completely satisfactory, and the results must accordingly be interpreted with caution. Certain features in our results, however, have been so marked and so consistent that we consider there can be

little doubt that they are the expression of true differences in toxicity. Where modifications in the amount of a toxicant which is incorporated in small quantities with an oil have resulted in graded increases or decreases of the kill obtained, we feel that there can be no reasonable doubt about this at all.

Among petroleum products, fuel oils are particularly suited for use as sprays on account of their fluidity and cheapness. We found, rather to our surprise, that a light distillate diesel fuel oil (exemplified by Shell "Dieseline", and the comparable products of other oil companies) appeared to be a good deal more effective than the darker-coloured fuel oils used for stationary diesel engines. In our tests the distillate diesel oil has always given kills comparable with those obtained with proprietary kerosene-base fly sprays of proved insecticidal value.

The tar-distillation products we have tested comprise crude naphtha, a high boiling range creosote (Messrs. Timbrol's special spraying creosote No. 259), and a "middle oil" of boiling range 200° to 240°C. (also ex Messrs. Timbrol's). The first appeared to be quite ineffective. The creosote and the middle oil both gave good results and appeared to have a toxicity roughly comparable with that of a distillate diesel fuel oil.

Experiments with mixtures of fuel oils and tar oils did not give clear-cut results; neither did our attempts to increase the toxicity of a distillate diesel fuel oil by the addition of insecticides such as carbon tetrachloride and dichlorethyl ether. We found, however, that the incorporation of 1–2 per cent. of dinitro-*o*-cyclohexylphenol (a compound that has been used to some extent—chiefly in America—in horticultural sprays) increased the toxicity of the diesel oil to a very marked extent. When an atomizing nozzle is employed, the use of an oil spray containing this toxicant results in very unpleasant working conditions, for dinitro-*o*-cyclohexylphenol is very irritating to the nose and throat; but if the mixture were applied with a nozzle which did not produce a mist-fine spray (as presumably would be the case in practice, where surfaces rather than spaces are being treated) we doubt whether much trouble would be experienced in this regard, particularly when treatments were being carried out in open premises.

The extent to which oils, rather than oil emulsions, can be used for the treatment of sheds and stacking sites must depend primarily on the availability of suitable spraying plants. The addition of a toxicant as effective as dinitro-*o*-cyclohexylphenol appears to be, would enable the dosage of oil to be reduced; but until the question of cost and availability has been clarified, it is impossible to say whether it would be justified in practice. This matter is being looked into, and experiments with other toxicants are proceeding.

Investigations on the Locust (Grasshopper) Problem.*

By K. H. L. Key, M.Sc., Ph.D. D.I.C.†

I assume that what the Council wishes to hear from me is an account of our work on the locust problem since its commencement five years ago, and the present position of the problem. I propose, therefore, to approach the subject from the historical point of view.

When the first grant for locust research was made in 1935, the problem presenting itself was roughly as follows: Extensive areas of agricultural country in New South Wales, Queensland, Victoria, South Australia, and Western Australia were liable to be over-run in almost any year by swarms of locusts or grasshoppers. These swarms sometimes appeared with hardly any warning, and did very severe damage. As an illustration of the possible extent of such damage, I may quote the late Mr. Gurney, then Chief Entomologist in New South Wales, who has estimated that in 1937-38 losses amounting to several million pounds were prevented in New South Wales by the expenditure of about £40,000 on control measures. At the time I am speaking of, it was not known how many species were concerned in these outbreaks, where the insects originated, nor what conditions led to their multiplication and migration. In the circumstances the only possible method of control was to wait until the flying swarms arrived, and then to poison the hoppers resulting from them.

Our investigations began with a taxonomic study of the insects concerned. It was found that there were two species of major importance, namely *Chortoicetes terminifera* and *Austroicetes cruciata*, as well as several others of minor importance. *Chortoicetes terminifera* is the main species in New South Wales and Queensland, and also occasionally infests Victoria and South Australia. It forms large, dense swarms having a range of migration of several hundred miles under suitable conditions. *Austroicetes cruciata* is a smaller insect which usually occurs in loose swarms only, and has a maximum range of about fifteen miles. In a recent mimeographed publication, non-technical illustrated descriptions have been given of these two species and of four others of minor importance.

We next proceeded to make a study of all past outbreaks of which records could be found in the literature or in the files of the State Agricultural Departments. It was found possible to distinguish the species concerned in the great majority of these outbreaks, and to draw conclusions of value regarding their regional and seasonal incidence. For example, the distribution of *Chortoicetes terminifera* and *Austroicetes cruciata*, both as scattered individuals and as swarms, has been worked out in some detail; the State of New South Wales has been divided into zones of varying susceptibility to infestation by

* An account given to a recent meeting of the full Council, Canberra, November, 1941.

† An officer of the Division of Economic Entomology.

Chortoicetes; and Western Australia has been similarly treated with regard to its susceptibility to *Austroicetes*. *Chortoicetes* has been shown to be capable of producing a succession of generations during the spring, summer, and autumn, but *Austroicetes* produces only a single spring generation.

The most valuable result of this study of past outbreaks, however, was the information it gave on the original source of the swarms composing an outbreak of *Chortoicetes*. In outbreak after outbreak it was found that the first swarms to be recorded were located in or near one or other of a limited number of relatively small regions situated mainly in central New South Wales. From these regions the flying swarms spread out, mainly in a south-easterly direction, to occupy much more extensive areas, but their ability to survive at swarming density in such areas was limited, and sooner or later they disappeared. The limited areas in which the swarms of a locust originate are known in the literature as *outbreak areas*. Their importance rests in the fact that they are fixed in position, can be fairly accurately demarcated, and constitute only a small fraction of the area which is liable ultimately to be invaded. Important outbreak areas are located in the Bogan and McQuarie country round Warren and Trangie, in the foothills of the Warrumbungle Mountains, in the Liverpool Plains, and in the Bland country round Lake Cowal. The results of this work on past outbreaks were published in the Council's Bulletin 117, which includes maps showing the location of the outbreak areas and suspected outbreak areas of *Chortoicetes*, and the relative susceptibility of various parts of New South Wales to invasion by swarms of this species.

At this stage an agreement was reached with the workers at the Waite Institute whereby we were to confine ourselves, as far as intensive work was concerned, to *Chortoicetes terminifera*, and they to *Austroicetes cruciata*. This was a logical step in view of the geographical range of the two species, and has prevented overlapping in the activities of the two institutions. Shortly after, at a conference of Commonwealth and State representatives concerned with the locust problem, the appointment to our staff of an additional assistant research officer, and of a technical officer, was recommended, and later carried into effect. At this conference it was also decided to adopt standard popular names for the two major species, the names chosen being the Australian Plague Locust for *Chortoicetes terminifera*, and the Small Plague Grasshopper for *Austroicetes cruciata*.

Encouraged by the results of our study of past outbreaks, we next approached the Agricultural Departments of New South Wales and Queensland with a scheme for the collection of more detailed and systematic information on locust distribution and movements than had hitherto been provided by their officers. This scheme, which is now in operation, was based on the experience of the International Centre for Locust Research in London, which for a number of years has been collecting information on locust migration from countries in Africa and Western Asia. It involved the creation of a Locust Information Service in each State (i.e., New South Wales and Queensland), administered by the State Departments of Agriculture. Information as to the distribution, stage of development, direction of migration, and so on, of swarms in the two States is recorded on maps by means of conventional signs, and these maps are submitted to Canberra monthly.

Working on these data, it has been possible to analyse the outbreaks of 1937-40 in considerable detail, and to correlate the distribution and movements of the locust swarms with meteorological data. It has been found that the life-cycle of *Chortoicetes* will only proceed without interruption and without high mortality as long as weather and pasture conditions, as determined by temperature and effective rainfall, remain within certain defined limits. These limits comprise a lower temperature limit, an upper moisture limit, and a lower moisture limit. When conditions move below the temperature limit, or outside the range of favourable moisture, either on the dry or on the wet side, the active stages (i.e., hoppers and fliers) die off. On the other hand, the eggs are able to survive periods of at least five months at temperatures below the threshold for hoppers and adults, and periods of about three months under conditions which would be too dry for hoppers and adults, and for hatching. A "climatic index" has been developed which can be used to express the climatic favourability of any given season from the point of view of locust multiplication.

The analysis of the data provided by the Locust Information Service has also confirmed the importance of the outbreak areas, and has enabled certain of them to be more accurately mapped than was possible before. It has also revealed regularities in the direction of migration of swarms of *Chortoicetes,* and possible explanations for these regularities.*

Extensive field surveys have been carried out in New South Wales and Queensland to study the nature of the country in the known outbreak areas. At the same time other regions with similar ecological characteristics have been located, and these must be suspected of being at least potential outbreak areas. Almost all the outbreak areas have been examined, most of them on more than one occasion. As a result of this work I was able to give a preliminary account of the essential features of the outbreak areas at the Locust Conference held in Melbourne in 1938. Our ideas on this question have now been considerably clarified and extended. . The essential features of the outbreak areas may be defined as follows: In the first place their climate is such that a relatively high proportion of seasons is favourable for multiplication of *Chortoicetes.* Almost all the outbreak areas are situated within the climatic zone in which the average season permits at least one generation to be passed through under favourable conditions. This climatic zone is a fairly extensive one, however, and the precise location of the outbreak areas within it is determined by other factors. A negative factor is found to be the presence of dense timber. Locusts will not enter dense timber, and frequently will not fly over it. Thus it is not surprising that all the outbreak areas are conspicuously lightly timbered.

Given a favourable climate and a relative absence of timber, the important determinants of the outbreak areas are their soil and drainage relationships. All the main outbreak areas which have been examined are situated on almost flat country, on which surface and subsoil drainage is necessarily poor. In the climatic belt we are considering, such country almost invariably develops a heavy grey or black self-mulching soil, either uniformly all over, or in the lower-lying areas.

* A paper is now being prepared for publication which will give a detailed account of these results.

It also shows a strong tendency to develop crab-holes, gilgais, and shallow watercourses of various types in which water collects after heavy rain. Wherever the country is not absolutely level, the slight rises are usually composed of a lighter-coloured compact soil bearing a sparse vegetation, and in many parts rapid alternations are found of relatively bare compact soil on the rises and heavy self-mulching soil, bearing a dense growth of grasses, in the depressions. It is this mixed type of country which we have come to recognise as typical outbreak area country. Its occurrence in New South Wales and Queensland has been mapped, and its limits, within the zone of favourable climate, coincide remarkably closely with the limits of the individual outbreak areas. Even outside the region of favourable climate, country of this type has been known to produce swarms in exceptionally favourable seasons.

In order to determine the precise significance in the biology of *Chortoicetes* of the various characteristics of an outbreak area which have just been described, we have undertaken an intensive study of one of the main outbreak areas in New South Wales from a field station at Trangie. The work involves periodic determinations of the locust population on selected sample areas, and parallel estimations of vegetation, soil, climate, and microclimate. The aim is to correlate fluctuations in the locust population with fluctuations in these environmental factors. We have found that the significance of the bare areas of compact soil is due to their suitability as oviposition sites, probably owing to the protection which soil of this type affords to the egg-pods deposited in it, in comparison with the unstable, friable surface of the self-mulching soil. The well-vegetated self-mulching areas, on the other hand, are important as food reservoirs in drought times, and also in providing shelter for adults and hoppers from extremes of heat and cold. For an area to permit of rapid multiplication of dispersed locusts, it is necessary for the two types of habitat to be present in the right proportion, and to be situated sufficiently close together for the insects to find their way without difficulty from one to the other.

A further important function of the well-vegetated areas, especially those situated in depressions, is probably that of providing the necessary conditions for a *concentration* of a scattered population into a small area, as distinct from the *multiplication* of the population. In dry times, locusts distributed over a considerable area congregate in the vegetated depressions. When brought together artificially in this way they are given an opportunity to develop that gregarious behaviour which will later cause them to keep together in swarms without outside aid, and to migrate out of the outbreak area. It has not yet been possible to study this phase of the problem directly, owing to the relatively low population which has been present in the area under investigation during the first two years of the work there, but important indications along these lines have been obtained.

You will have noticed that in the work I have been describing our attention has narrowed down from extensive surveys of past outbreaks, and of large parts of New South Wales and Queensland, to an intensive study of a selected part of a single outbreak area. The final stage in this narrowing-down process is represented by the laboratory work we are carrying out here in Canberra. Locusts are being reared from hatching to death at various combinations of temperature and relative

humidity, the object being to determine the rate of development, length of life, fecundity, and mortality, under a wide range of controlled conditions. Experiments in which the food factor is varied will be undertaken later. Since population fluctuations in the field are fundamentally due to variations in rate of development, fecundity, and mortality, while differences in soil, vegetation and climate can be referred to temperature, moisture and food, these experiments should provide the basic explanation of the relationships observed in the field.

We may now enquire what practical results have followed, or could follow, from the conclusions already reached in the locust research. It seems to me that there are three main directions in which practical application of the results can be expected:—

In the first place, our knowledge of the location of the outbreak areas, and of the normal course of outbreaks, enables rough forecasts to be made, both of developments from month to month during an outbreak, and also of when outbreaks are likely to arise. Tentative forecasts along these lines have been prepared for the past eighteen months, and issued to a limited number of interested parties. The accuracy of such forecasts could be much increased if the outbreak areas could be patrolled at intervals.

Secondly, knowledge of the location of the outbreak areas should enable swarms or incipient swarms to be controlled by the usual poisoning methods either before they leave the outbreak areas, or before they have moved far from them. It may also be possible to devise a modified poisoning technique which could be used to keep the population permanently well below the swarming density. Our present staff is not sufficient to enable experiments to be carried out along these lines.

Finally, our increasing knowledge of the relation of the vegetation and soil of the outbreak areas to the life of the locust should ultimately enable us to recommend measures for rendering these areas ecologically less favourable to it, thus attacking the problem at its root. In theory, if either the bare compact areas or the well-vegetated areas could be changed in such a way that they lost their essential characteristics, the problem would be solved. The difficulty would be to bring about the necessary changes economically, and within the framework of approved grazing practice. It is probable, however, that by modifications of pasture management, including the use of the plough on bare areas, and by the afforestation of limited critical areas, a great improvement might be effected. If a fodder tree were used on the areas requiring afforestation, some of the objection to the withdrawal of land for this purpose might be overcome. It would no doubt still be necessary to use poison bait in places to control the early stages of swarming.

There seems to me a definite possibility that the systematic application of these measures, in combination, may in time enable us to prevent plagues of locusts from arising. However, a great deal more research is needed before definite recommendations could be made. So far nothing at all has been done along these practical lines, and nothing could be attempted without an increase in the staff engaged on the problem. At present this staff comprises two assistant research officers, a technical officer, and a laboratory assistant.

To sum up, I think we may say that the phase of the work involving extensive field surveys and the studying of masses of data collected by others, is over. We have settled down to intensive field and laboratory investigations of a limited number of crucial problems. The solutions of these should be obtained within about two years, and we will then be able to turn our attention to the testing out of practical control measures, the general nature of which can already be forecast. I would like to point out that we have tackled the locust problem in this country with a considerably smaller research staff than is employed on any other economically important species of locust I can think of. Under the circumstances I consider that our rate of progress has been satisfactory, and in certain respects our conclusions will be more soundly based than those on other species. We have now reached the stage where the problems still requiring investigation are perfectly clearly defined, and our rate of progress would be strictly proportional to the size of our staff.

Oriental Peach Moth Investigations.

General Statement, July, 1941.

The Oriental peach moth is a serious pest in the Goulburn Valley, Victoria, and attacks in particular the late canning peaches which are an important product of the district. Its spread was insidious, but by 1934 growers had become alarmed and a thorough investigation of the pest was arranged. Funds were made available jointly by the Canned Fruits Control Board and the Commonwealth Bank (through its Rural Credits Development Fund), and these two bodies have since shared the whole cost of the investigation which has been undertaken by the Council and the Victorian Department of Agriculture in co-operation. The investigator was supplied by the Council, and the Department administers the finances and helps in other ways. In the season 1940-41, the Northern Fruitgrowers' Association also contributed £50 towards the cost of the work. The investigation was carried out under the general supervision of an Advisory Committee representative of the Department, the Canned Fruits Control Board, and the Council. Results of the first season's work, covering the period 1934-35, were reported in this Journal (8: 131, 1935), and the results of the work during 1935-38 were summarized in the Council's Pamphlet No. 88.—Ed.

1. Investigations During the Past Season.

The main object of the past season's work was to determine whether the parasite *Macrocentrus ancylivorus* had become established in the Goulburn Valley, and if so to determine its distribution and abundance. No further parasites were introduced from the U.S.A. as it was considered that the number of parasites introduced and liberated during the previous five years was more than ample to ensure their establishment if conditions in this country were favourable.

The survey for parasites was somewhat hampered by the fact that the population of the Oriental peach moth during the season 1940-41 was the lowest on record, so that considerable difficulty was often

experienced in finding more than a small number of infested peach shoots. However, during the season some 8,400 infested shoots were gathered, and from these 3,838 mature moth larvae were obtained. From these 3,220 moths emerged. In the course of this work, only one specimen of *Macrocentrus ancylivorus* was bred from shoots collected in the field, and this was from the first generation of peach moth larvae.

The only parasites liberated during the season were 20 males and 20 females of *M. ancylivorus* which were released at Bamawm Extension.

Further observations on the life cycle of the Oriental peach moth (*Cydia molesta*) were also made during the past season.

2. General Review of Oriental Peach Moth Investigations.

When the investigation was begun in 1934, attention was naturally directed mainly to the study of the life cycle and behaviour of the peach moth, and this study has been continued right up to the present time. It has been shown that the Oriental peach moth has the following attributes which render it difficult to control by chemical means:—

(*a*) The larvae remain buried in plant tissue for practically the whole of their life.

(*b*) When boring into a shoot or fruit the larvae excavate a hole with their jaws but do not swallow any of the plant tissue until the burrow is sufficiently large to hold the whole larva.

(*c*) The insects pupate in silk cocoons which cannot be penetrated by ordinary sprays.

(*d*) The moths are not sufficiently readily attracted to light or aromatic substances to make it possible to control them effectively by means of traps.

(*e*) The peach moth passes through five generations per year and multiplies so rapidly under favourable conditions that it can counteract an earlier reduction to a low density by increasing to a high density in a short space of time.

During 1934 to 1938 much attention was given to the possibility of using insecticides against the Oriental peach moth. All types of insecticides that might conceivably be of use against a pest of this kind were tried under laboratory conditions, and of these the ones that gave promising results were tried in fully replicated field experiments. Unfortunately no spray gave worth-while results under field conditions. Exactly the same lack of success attended the efforts of investigators in the U.S.A. There appear to be no further insecticides known at present that are worth trying, but if any new type of insecticides is developed which offers any prospect of controlling such a pest as the peach moth, it will naturally be tested here.

The first introduction of parasites was made in 1935, being sent by the courtesy of the U.S. Department of Agriculture. In 1937, Mr. G. A. H. Helson visited the U.S.A. to obtain Oriental peach moth parasites for the Council and to study the breeding technique developed

at the laboratory of the U.S. Department of Agriculture at Moorestown, N.J., and other places. In 1938 and 1939 an American entomologist temporarily employed by the Council bred parasites at Moorestown for sending to Australia. The following table shows the number of parasites liberated in the Goulburn Valley in each season since the Oriental Peach Moth Committee was formed.

Parasite.	Season.						Total Liberated.
	1935–36.	1936–37.	1937–38.	1938–39.	1939–40.	1940–41.	
Macrocentrus ancylivorus	62	1,138	2,482	3,412	4,952	54	12,100
Macrocentrus delicatus ..	..	..	..	133	..	..	133
Bassus diversus ..	..	..	843	102	..	..	945
Inareolata molestae ..	..	..	42	36	..	..	78
Ascogaster carpocapsae ..	..	..	164	2	..	..	166
Glypta rufiscutellaris ..	41	831	267	..	22	..	1,161
Total	103	1,969	3,798	3,685	4,974	54	14,583

In the course of this parasite introduction work it soon became evident that the only species showing any promise of being successful in this country was *Macrocentrus ancylivorus*. In 1937, comparatively small numbers of this parasite liberated early in the growing season of the peach increased so greatly that in 1937-38 some 60 per cent. of the peach moth larvae in the orchards where the liberations had been made were attacked by *M. ancylivorus*. This showed that conditions in the Goulburn Valley during the warmer months of the year are quite favourable for this parasite. Owing first to drought conditions and later to floods, the season 1938-39 was most unfavourable for the peach moth and its parasites, and when following this in the spring of 1939 seven parasites were recovered from the first generation of peach moth larvae, hopes were entertained that *M. ancylivorus* after all might find the winter conditions favourable here. Unfortunately, in the season 1940-41, which followed a good season during which large numbers of *M. ancylivorus* had been liberated, only one specimen of this parasite was recovered from the field in spite of a very intensive search. There seems little doubt, therefore, that the late autumn and winter period is unfavourable for *M. ancylivorus* in the Goulburn Valley. There is strong evidence to suggest that this is due to the fact that the greater part of the parasites which develop in the last generation of peach moth larvae do not remain quiescent throughout late autumn and winter like their hosts, but emerge as adults in late autumn when there are no hosts to attack and so perish without leaving offspring. In those parts of the U.S.A. where *M. ancylivorus* exercises an effective control of the Oriental peach moth, this parasite has an important alternative host, the strawberry leaf roller (*Ancylis comptana*), which maintains its abundance at times when peach moth larvae are scarce or absent.

An attempt was made to develop a technique for breeding *M. ancylivorus* on a large scale with the ultimate object of breeding sufficiently large numbers under laboratory conditions to permit of the liberation each spring of effective numbers of parasites. Unfortunately it was not found possible to substitute for fresh peach shoots anything that would be suitable for the breeding of the host moths in large numbers.

3. Present Position and Future of Investigations.

From the foregoing review it will be seen that—

(*a*) The Oriental peach moth has special habits which render it very difficult to control by means of insecticides.

(*b*) Until some new type of insecticide is evolved nothing worth while remains to be done in the direction of testing insecticides against the Oriental peach moth.

(*c*) All the known parasites of the Oriental peach moth which seemed to give any promise of control have been tested in Australia. In spite of the fact that far more parasites have been liberated here, and the process of introduction has been persisted in for a longer period than is usual when attempting to establish a parasite from another country, no evidence has yet been obtained that any one of the parasites has become established here.

(*d*) The artificial maintenance of *M. ancylivorus* in sufficiently large numbers to be used for effective control of the Oriental peach moth in the field has not proved possible.

It appears, therefore, that the only things remaining to be done are:—

(*a*) To maintain a survey in the Goulburn Valley during the next few years to see whether, after all, one of the parasites may have become established and to note if it increases in abundance.

(*b*) To keep in touch with insecticide developments, particularly those concerning Oriental peach moth or any similar pests, and to test any new insecticide or method which offers any promise.

(*c*) To keep in touch with other organizations such as the U.S. Department of Agriculture, which are concerned with the Oriental peach moth.

(*d*) To introduce any newly discovered parasites of the Oriental peach moth which are shown to have any promise.

NOTES.

The Results of Inoculating Grape Vines with a Fungus Isolated from "Dying Vines".

(Contributed by W. V. Ludbrook, B.Agr.Sc., Ph.D., and D. V. Walters, M.Agr.Sc.†)*

A trouble termed "dying vines" by Murray Valley grapegrowers has been under observation by the writers since 1938, and was described by them in 1940.‡ An unidentified fungus was isolated from affected vines. During 1938, the following healthy sultana vines were inoculated with this fungus:—Twelve vines (2 years old) in pots at Canberra, 30 vines (17 years old) at Merbein, and 23 vines (15 years old) at Woorinen. Agar inoculum was introduced under aseptic conditions into wounds in the stems just below ground level, which were then plugged. Sterilized inoculum was similarly introduced into an equal number of control vines at each station.

Six inoculated and six control vines at Canberra were uprooted and examined in 1939. They all appeared normal except for slight brown discoloration close to the point of inoculation, as did two vines from Woorinen examined in 1940 (loc. cit.). Two inoculated and two control vines at Canberra gradually became very stunted, dying in 1941. This was possibly due to their having become too large for the containers in which they were growing; two other inoculated vines and one control became slightly stunted. At Woorinen, eight of the 23 inoculated vines became slightly, and 3 markedly, stunted; of the 23 controls, 4 were slightly and 1 markedly stunted. This difference was not statistically significant. The gradual stunting exhibited by these vines was not characteristic of naturally occurring "dying vines"; death is usually the first visible symptom of this trouble, which is not ordinarily preceded by stunting. The writers' inoculations failed to reproduce this condition. The other vines at Canberra and Woorinen, and all those at Merbein, remained healthy during the four seasons which have elapsed since they were treated.

The surviving vines at Canberra, and also six from Merbein and eight from Woorinen, were uprooted and examined in December, 1941. On splitting the trunks, the wood close to the point of inoculation was found to have developed a brown discoloration similar to that observed in "dying vines", but much less extensive. In no case did this discoloration extend for more than 3 cm. from the point of inoculation, and usually not for more than ½ to 1 cm. There was no consistent difference in size of discolored area between inoculated and control vines, and the fungus used for inoculation was isolated from about the same percentage of vines in each of the two groups. Various other imperfect fungi and bacteria also developed from platings of discolored wood, but the above-mentioned fungus occurred much more often than any other organism. It was isolated more consistently from the Woorinen vines than from those grown at Merbein or Canberra.

These results are thought to confirm the opinion previously expressed, that the trouble termed "dying vines" is primarily physiological, and that the fungus used for inoculation, though commonly found in affected wood, is only weakly (if at all) parasitic. The investigation was not

* An officer of the Division of Plant Industry.
† An officer of the Commonwealth Research Station, Merbein.
‡ Walters, D. V., and Ludbrook W. V.—"Dying Vines" in the Murray Valley. *J. Coun. Sci. Ind. Res. (Aust.)*, **13**: 183-186 (1940).

intensive enough to exclude the possibility of a pathogen being involved, or to elucidate the environmental factors possibly concerned. However, as the writers are unable to pursue the subject further, it is thought advisable to present the results obtained. There has not been any obvious increase in the economic importance of the trouble since the previous paper appeared.

A Mechanical Device for the Spread of Disease Agents Amongst Rabbits.

A trap has been devised to aid in the distribution of viral and other disease-producing agents amongst rabbits, should it be desirable to use these agents for the control of rabbit populations. An ordinary standard spring-type steel rabbit trap has been adapted for the purpose and the adaptions are illustrated in Figs. 1 and 2; they were devised by officers of the Council's Division of Animal Health and Nutrition.

The alterations and additions involved are outlined below:—

(i) The eye (A, Fig. 1) at the end of the spring that closes the two jaws of the trap when released, is cut away so that only one jaw of the trap will be active. As it is not intended to catch the rabbit with this modified trap, only one jaw should be brought into operation by the spring.

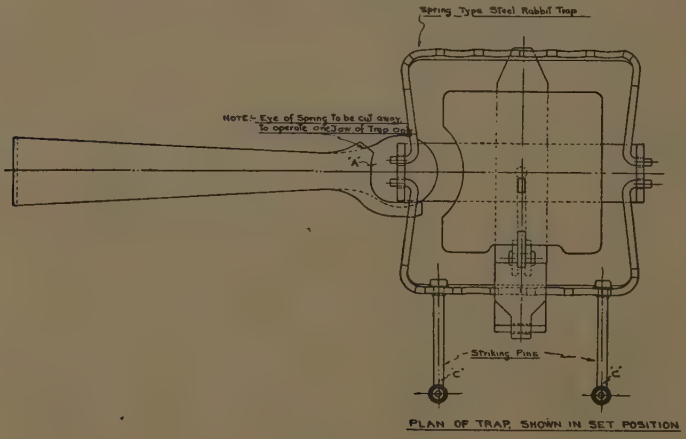

Fig. 1.

(ii) A small mild steel block (B, Fig. 2) is welded to the under-side of the release catch so as to allow the active jaw of the trap to be pressed to a lower position than under normal conditions; this is done to keep the

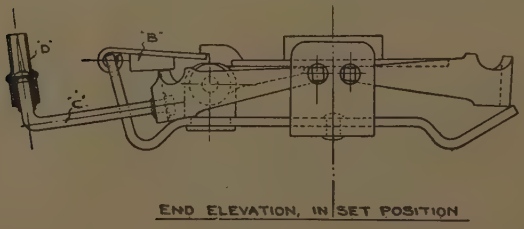

Fig. 2.

inoculating pin in a lower position. As the whole trap and striking pins requires to be covered with a thin layer of sand or soil when properly set, a small piece of thin paper is used to prevent the sand from getting under the trip plate, and small discs of thin waxed cardboard are used to cover the inoculating pins, and prevent the sand from getting into the virus container when covered with the sand or soil.

(iii) The two striking pins (C, Figs. 1 and 2) are welded to the active jaw of the trap. A short piece of rubber tubing is fitted over the end of these pins for insulating purposes.

(iv) The two inoculating pins (D, Fig. 2) consist of No. 22 short cartridge cases, or similar brass cases, with a ½-inch long steel tack soldered to the top of the cases. Pieces of soft rubber tubing are fitted over the brass cases and cut off level with the point of the tack, thus forming suitable virus containers. In order to keep the tacks covered with the virus, these inoculating pins are fitted over the rubber sleeves on the end of the striking pins (C).

Recent Publications of the Council.

Since the last issue of the *Journal,* the following publications of the Council have been issued:—

Bulletin No. 143.—"Production of Dried Grapes in Murray Valley Irrigation Settlements. 1. Viticulture," by A. V. Lyon, M.Agr.Sc., and D. V. Walters, M.Agr.Sc.

This Bulletin gives the results of investigations conducted at the Viticultural Research Station, Merbein, over a long period during which various branches of the industry have afforded considerable financial and other assistance. The present publication is confined to viticulture in the Murray Valley irrigation settlements; at a later stage it is proposed to issue separate bulletins dealing with irrigation and drainage of the vine and with the processing of the fruit.

The development of the vine under normal conditions has been carefully studied at the Station so that any variation caused by climate, pruning, trellising, etc., could be examined. The effects of these different factors are given in some detail. Among other findings, a relationship has been shown between amounts of foliage and fruit when maintaining quality, and the effects of winter and summer pruning in this connection are discussed. The vine is capable of a marked degree of compensation within fairly wide limits, but it is possible to reach a stage where increase in bearing units (spurs in the Zante currant and canes in the sultana) is not of benefit. It has also been shown that examination of the shoots in early spring, in order to record the percentage of fruitful and barren shoots, is a reliable method of estimating the amount of the crop some months later.

It has been shown that nitrogen is the most important fertilizer element and although spectacular results are not possible by any system of manuring, the manurial programme should include the application of nitrogen and the preservation of soil fertility by the use of green manure, such as tick beans.

Pamphlet No. 109.—"Studies on the Physiology and Toxicology of Blowflies. 8. Rate of Ammonia Production by Larvæ of *Lucilia cuprina*

and its Distribution in this Insect. 9. The Enzymes Responsible for Ammonia Production by Larvæ of *Lucilia cuprina*," by F. G. Lennox, M.Sc., A.I.C.

This pamphlet is the fourth of a series dealing with the physiology of the sheep blowfly and its reactions to poisons. The work forms one of the two main lines of attack being made on the blowfly problem by the Council; the other is the study of the factors that influence the susceptibility of sheep to blowfly strike.

In contrast with most insects, which excrete the bulk of their nitrogenous waste in the form of uric acid and its salts, blowfly larvæ eliminate most of their nitrogen as ammonia, which involves the loss of no unburnt carbon. Furthermore, another very simple end-product. carbon dioxide, serves to neutralize the alkali before excretion. In these particulars the metabolic efficiency of these organisms is of a very high order, and this no doubt partly accounts for their ability to grow on very little food.

Forthcoming Publications of the Council.

At the present time the following future publications of the Council are in the press:—

Bulletin No. 142.—"A Soil and Land Use Survey of the Hundreds of Riddoch, Hindmarsh, Grey, Young, and Nangwarry, County Grey, South Australia," by C. G. Stephens, M.Sc., A.A.C.I., R. L. Crocker, M.Sc., B. Butler, B.Ag.Sc., and R. Smith, B.Ag.Sc.

Bulletin No. 144.—"Interference in a Wind-Tunnel of Octagonal Section," by G. K. Batchelor, M.Sc.

Bulletin No. 145.—"Friction and Lubrication. Report No. 1.—The Theory of Metallic Friction," by F. P. Bowden, Sc.D. (Cantab.), and D. Tabor, Ph.D. (Cantab.).

Bulletin No. 146.—"An Analysis of the Outbreaks of the Australian Plague Locust (*Chortoicetes terminifera* Walk.) during the Seasons 1937-38 and 1938-39," by K. H. L. Key, M.Sc., Ph.D.

Bulletin No. 147.—"Enzootic Ataxia and Copper Deficiency of Sheep in Western Australia," by H. W. Bennetts, D.V.Sc., and A. B. Beck, M.Sc.

Bulletin No. 148.—"Studies in Fertility in Sheep. II. Seminal Changes Affecting Fertility in Rams," by R.M.C. Gunn, D.V.Sc., B.Sc.Agric., M.R.C.V.S., R. N. Sanders, B.V.Sc., and W. Granger, B.V.Sc.

Pamphlet No. 110.—"The Main Virus Diseases of the Potato in Victoria," by J. G. Bald, M.Agr.Sc., Ph.D., and A. T. Pugsley, B.Agr.Sc.

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Pamphlet No. ?.—"Plant Introduction. 1. A Review, with Notes on Outstanding Species," by A. McTaggart, M.S.A., Ph.D. "2. Preliminary Selection of Pasture Species at Lawes," by T. B. Paltridge, B.Sc.

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